CHAPTER

8

Differential Effects of EGF, TGF-β and NGF on Tail Regeneration.

Growth factors are customarily implicated in growth processes and developmental phenomena as they are powerful evokators of cell proliferation and differentiation. The ability to respond to particular growth factors can be an important developmental event as an interplay of a cascade of growth factors may be the key to orderly progession of developmental processes. Regeneration of vertebrate appendages which represents a recapitulation of early ontogenic process in a restricted sense affords excellent opportunities to explore the role of growth factors. Except for the studies on firbroblast growth factor (bFGF) on lens regeneration in newts (Cuny *et al.,* 1986; Gospodarowicz, *et al.,* 1987) and limb regeneration in adult frogs (Gospodarowicz and Mescher, 1981), no sutides have addressed to the possible influence of other growth factors and, growth factors in general on regeneration. Hence in the present study, the influence of exogenous administration of Nerve Growth Factor (NGF), Epidermal Growth Factor (EGF) and Transforming Growth Factor-β (TGF-β) has been assessed on the course of tail regeneration in the lizard, *Hemidactylus flaviviridis*. Of the three growth factors tested, only NGF gave favourable response. Due to the importance of both thyroxine and NGF in the development of nervous system and as thyroxine is shown to increase NGF content (Walker *et al.,* 1979), the relationship between the two has also been evaluated by studying the influence of NGF given to hypothyroid lizards.

MATERIAL AND METHODS

Healthy adult lizards *(Hemidactylus flaviviridis)* procured from the local animal dealer weighing 10-12 grms and having a snout-vent length of 8-10 cms were used for the experiments. The lizards were acclimatized to the laboratory conditions for a week before being brought into the experimental set-ups. The study consisted of two parts;

PART I : Evaluation of the influence of NGF, EGF and TGF-β.

A total of 40 lizars were divided into 4 groups of 10 each.

GROUP I : [CONTROL]

The tail of these lizards was autotomised three segments distal to the vent and injected with 0.6% saline at the cut end of the tail for 15 days.

GROUP II : [NGF]

The tail of these lizards was autotomized three segments distal to the vent and injected with 10 ng of NGF in 0.1 ml saline at the cut end of the tail for three consecutive days starting from the day of autotomy.

GROUP III: [EGF]

The tail of these lizards was autotomized three segments distal to the vent and injected with 5 ng of EGF in 0.1 ml saline for 10 consecutive days starting from the day of autotomy.

GROUP IV: [TGF-β]

The tail of these lizards was autotomized three segments distal to the vent and injected with 5 ng of TGF-β in 0.1 ml saline for 10 consecutive days starting from the day of autotomy.

PART II : Evalualtion of the influence of hypothyroidism, thyroxine replacement or replacement with NGF.

A total of 40 lizards were divided into 4 groups of 10 each.

GROUP I : [CONTROL]

This group of lizards served as euthyroidic controls. They were force fed 0.6% saline on every alternate day. At the end of the 5th dose of saline, the tail was autotomized. Saline feeding was continued till the end of the experiment.

GROUP II : [HYPOTHYROID - HT]

These lizards were rendered hypothyroidic by force feeding 0.1% 6-propyl, 2-thiouracil (PTU). The pH of the solution was adjusted to 8-8.2. The tail of these lizards was autotomized at the end of the 5th dose of PTU and PTU feeding was continued every alternate day till the end of the experiment.

GROUP III : [Thyroxine Replacement - TR]

These lizards were also rendered hypothyroid as those of Group II and the tail autotomized at the end of the 5th dose of PTU. Subsequent to autotomy PTU feeding was continued every alternate day. Apart from this, they were also injected with 10 μg of thyroxine in 0.1 ml saline at the cut end of the tail every alternate day starting from 3 days post-autotomy and continued till the end of the experiment.

GROUP IV : [NGF replacement : HT : NGF]

These lizards as those in Group II were also rendered hypothyroid and the tail autotomized at the end of the 5th dose of PTU. Five injections of NGF (10 ng/0.1 ml saline) were given *in loco* at the cut end of the tail for 5 consecutive days post-autotomy. PTU feeding was continued till the end of the experiment.

NGF and EGF were purchased from Sigma Chemicals, U.S.A., while TGF-β was a gift from the Department of Endocrinology, PGIBMS, Madras.

Parameters Evaluated

Subsequent to treatment with NGF, EGF and TGF-β, the number of days taken to reach the various arbitrary stages of regeneration like, wound healing (WH),

preblastema (PB), blastema (B) and initiation of growth (IG) was noted. Since there was inhibitory influence of EGF and TGF-β, the tails of these lizards were cut and processed for histological observations. Prior to dehydration and embedding in paraffin, the tails were decalcified and sections of 3-5μ and 8-10μ thickness were cut and stained in Casson's - or Masson's trichome. Some sections were also stained by the PAS technique. Since NGF treatment had a stimulatory influence on regeneration, the length of tail regenerated in control, NGF, HT, TR and HT: NGF groups of lizards was measured every alternate day. The growth rate per day and the total percentage of tail replaced were also calculated. The data was subjected Student's t-test and to Duncan's multile range test (Duncan, 1955).

RESULTS

EGF and TGF-β treatment (Table I). Treatment with both EGF and TFG-β resulted in inhibition of regeneration. Though EGF promoted earlier would healing, the attainment of preblastema and blastema stages were delayed. In contrast, though TGF-β did not show any effect on WH, the formation of PB and B stages was significantly delayed and a poor blastema was formed. However lizards, injected with TGF-β after the formation of a blastema did not show any effect and they regenerated their tail at the same rate as the controls. (Plate-8.1 and 8.2)

TABLE - 1

Number of days taken to attain various arbitrary stages in control and experimental lizards.

Manipulations	WH	PB	B	IG
CONTROL	7	9	10	11
EGF	6	11	13	17
TGF-β	7	13	17	-
NGF	5	6	7	8

WH - Wound healing, PB - Preblastema, B - Blastema, IG - Initiation of growth, EGF - Epidermal growth factor, TGF- β - Transforming growth factor, NGF - Nerve growth factor, T4 - Thyroxine.

Table - 2

Number of days taken to attain various arbitrary stages in control and experimental lizards.

Manipulations	WH	PB	B	IG
CONTROL	7	9	10	11
NGF	5	6	7	8
PTU	10	11	13	14
PTU + T4	9	10	11	12
PTU + NGF	8	9	10	11

WH - Wound healing, PB - Preblastema, B - Blastema, IG - Initiation of growth, EGF - Epidermal growth factor, TGF- β - Transforming growth factor, NGF - Nerve growth factor, PTU - 6-propyl-thiouracil, T4 - Thyroxine.

LEGENDS TO FIGURES

Plate - 8.1

A & B : Photographs of lizards treated with 5 ng EGF *in loco* at the end of 15 days.

A : EGF treated lizard showing a lethargic blastema formation.

B : Close up view of the same.

PLATE - 8.1

A

B

LEGENDS TO FIGURES

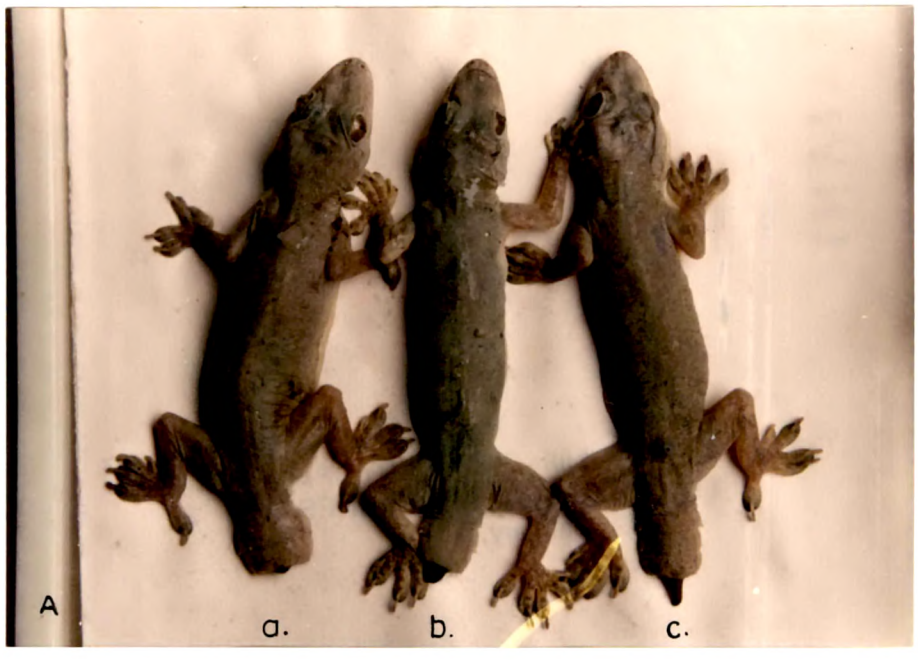
Plate - 8.2

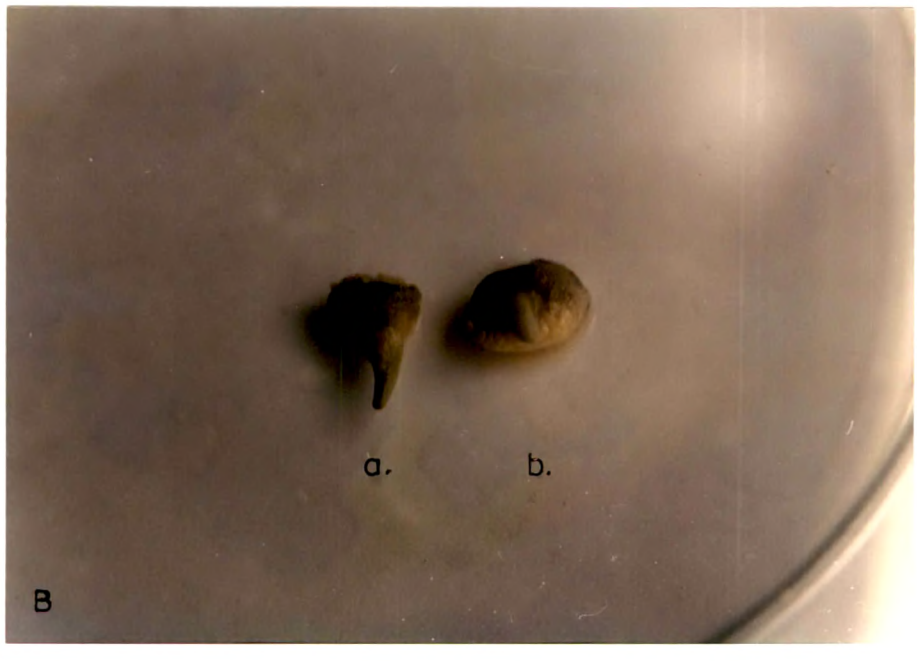
A & B : Photographs of lizards of control and EGF treated lizards.

A : Photographs of control (c), 1 ng EGF treated (b) and 5 ng EGF treated lizard (a), showing the dose dependent inhibitory influence of EGF at the end of 15 days.

B : Severed off tail stumps of control (a) and 1 ng EGF treated lizards (b) Note the hooked regenerate in b.

PLATE - 8.2





Histological Observations

Histologically, the EGF treated lizards showed thickened blastemic epithelium with tendency for keratinization and scale formation. In many cases, specific staining indicated formation of collagen below the blastemic epithelium and even in the mesenchymal mass (Plates 8.6 and 8.7). Precocious and pre-mature differentiation of muscle elements could be clearly seen even extending upto the distal tip close to the apical epithelium (Plate 8.10) . Lizards given only 1ng EGF or even in those lizards which were given 5 ng EGF where some growth occurred after the stoppage of EGF treatment, tended to show a hooked growth and in these cases PAS staining indicated less GAG substances in the differentiating cartilaginous tube (Plates 8.9, 8.10). TGF-β treatment seemed to affect muscle dedifferentiation (Plate 8.14). Erythropoeitic activity was greatly stimulated in the adipose tissue of the stump tail which got accumulated in large numbers at the cut end below the eptthelium (Plates 8.14 - 8.16). Differential staining revealed increased GAG deposition and precocious and preponderant chondrogenic differentiation (Plates 8.16, 8.17). Muscle differentiation was totally inhibited.

Control Plate - 8.3 - 8.5

NGF Treatment : (Figs 1-3 and tables 2-4)

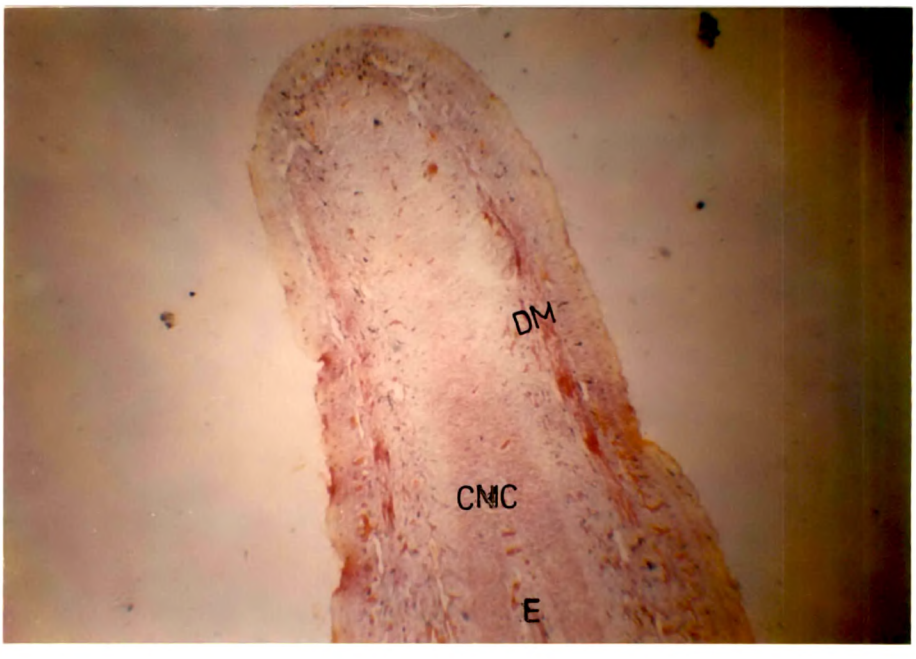
NGF treatement resulted in faster wound healing and early blastema formation and initiation of growth. There was sustained faster rate of growth resulting in 20% replacement of lost tail within 25 days as compared to 14% in the control lizards.

LEGEND TO FIGURE

Plate - 8.3

Photomicrograph of the top of regenerating tail of a control lizard showing progression of histodifferentiation proximodistally (45 X). Mallory's triple staining. Note the differentiating muscle (DM) appearing red in colour and cartilaginous neural canal (CNC) with ependyma (E) within.

PLATE - 8.3



LEGENDS TO FIGURES

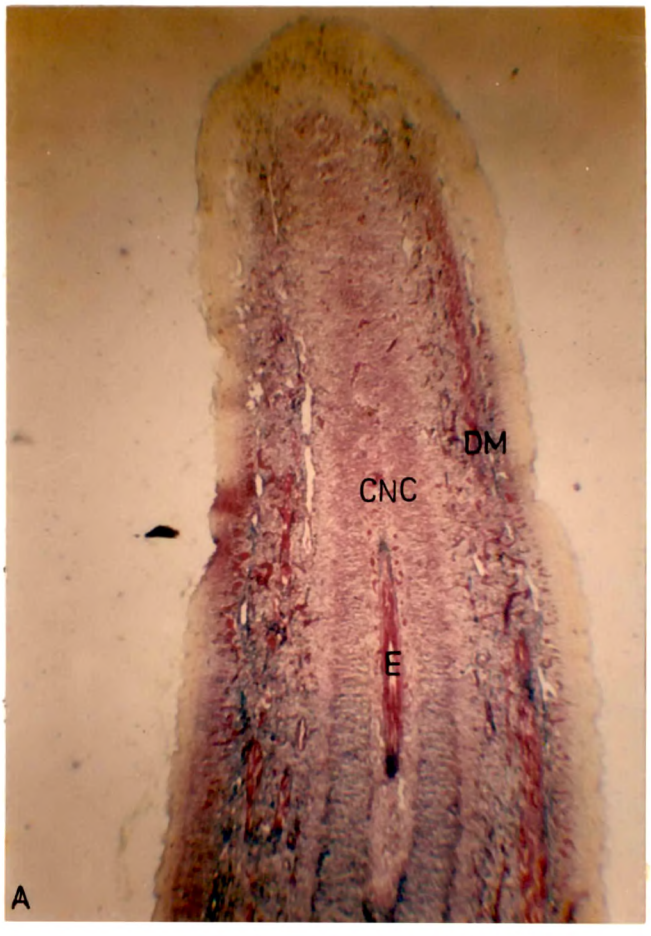
Plate - 8.4

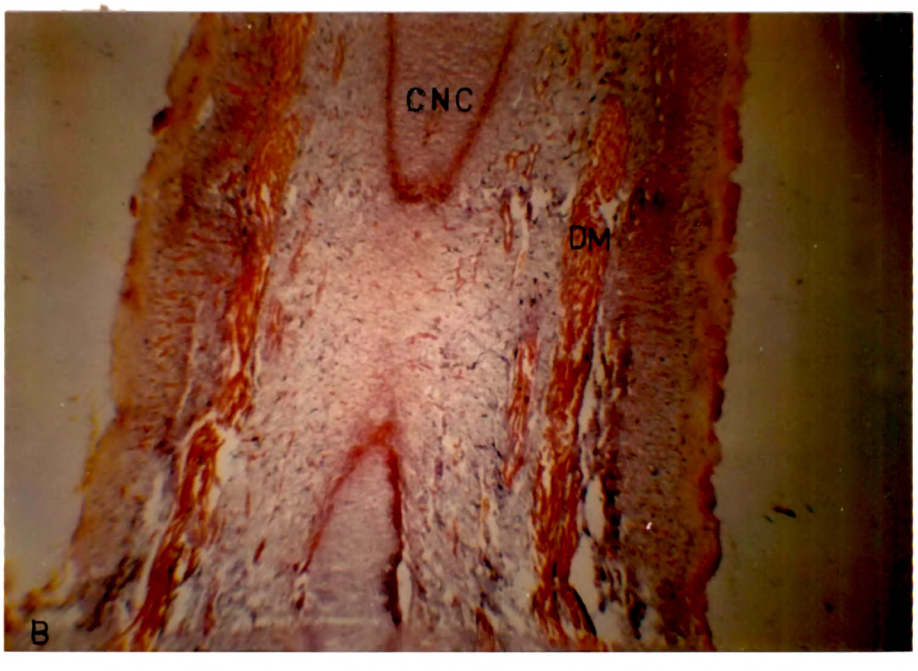
A & B : Photomicrograph of a section of a regenerating tail of a control lizard in the differentiation stage showing various differentiating elements.

A : Proximal and

B : Distal parts (45 X) Casson's stain.
Note the differentiating muscles (DM) orange in colour, cartilaginous neural canal (CNC) in the centre collagen appears blue.

PLATE - 8.4





LEGENDS TO FIGURES

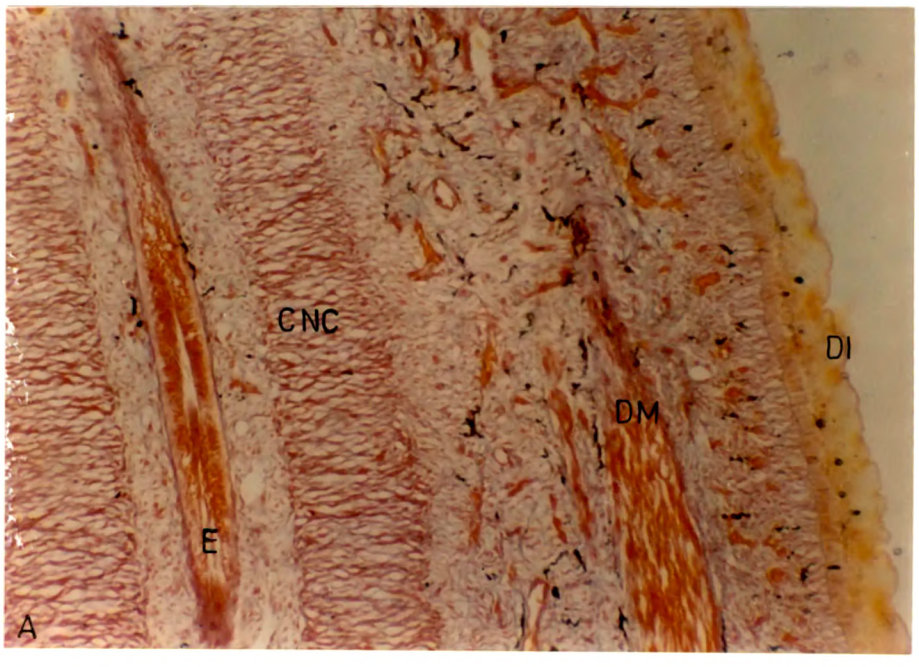
Plate - 8.5

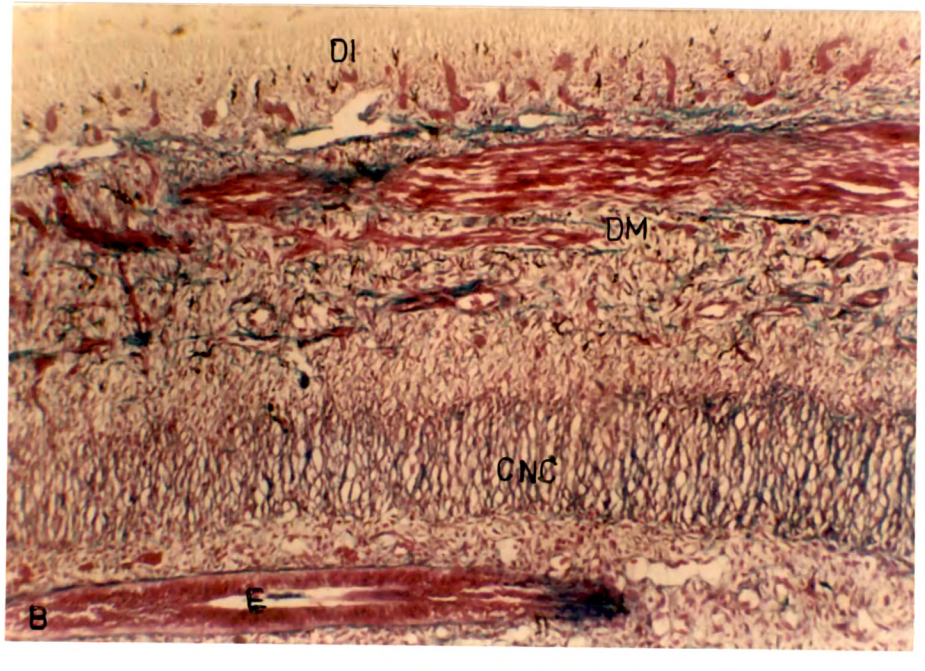
A & B : Photomicrographs of section of regenerating tail of a control lizard in the differentiating stage (150 X).

A : Enlarged version of Casson's stained section showing the lateral half with differentiating muscle (DM), differentiating integument (DI), cartilaginous neural canal (CNC) and ependyma (E). Integument appears yellow, muscle and ependyma appears orange and cartilaginous neural canal appears pink.

B : Central part of section of differentiating tail stained with Mallory's triple stain showing differentiating integument (DI), differentiating muscles (DM), cartilaginous neural canal (CNC) and ependyma (E). Differentiating muscles and ependyma appears red and collagen appears green.

PLATE - 8.5



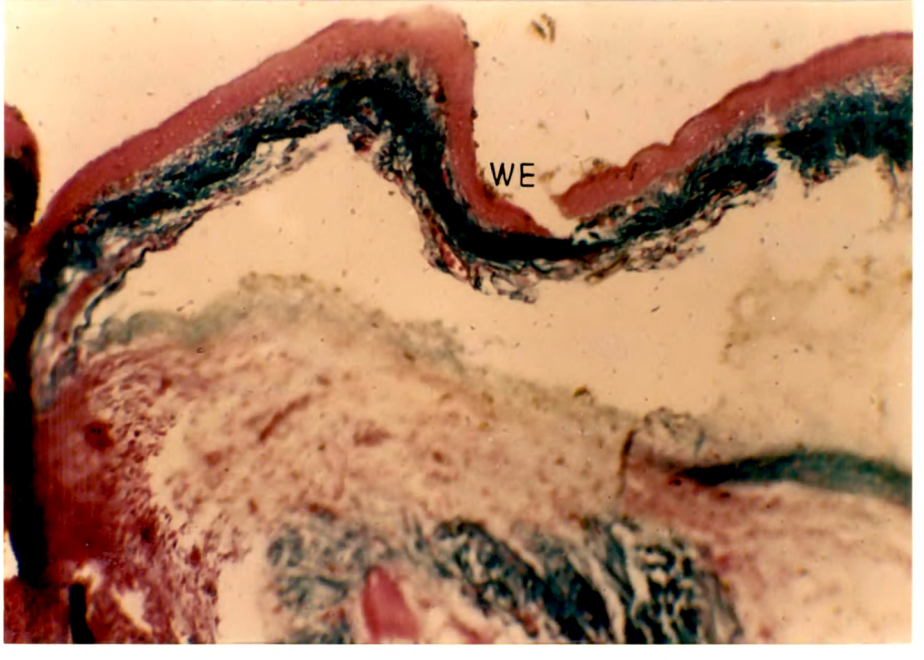


LEGEND TO FIGURE

Plate - 8.6

Photomicrograph of the section of the tail of experimental lizards treated with EGF in the wound healing stage (145 X). Mallory's triple staining. Note the deposition of a thick layer of collagen (green) below the thickened wound epithelium (WE).

PLATE - 8.6



LEGENDS TO FIGURES

Plate - 8.7

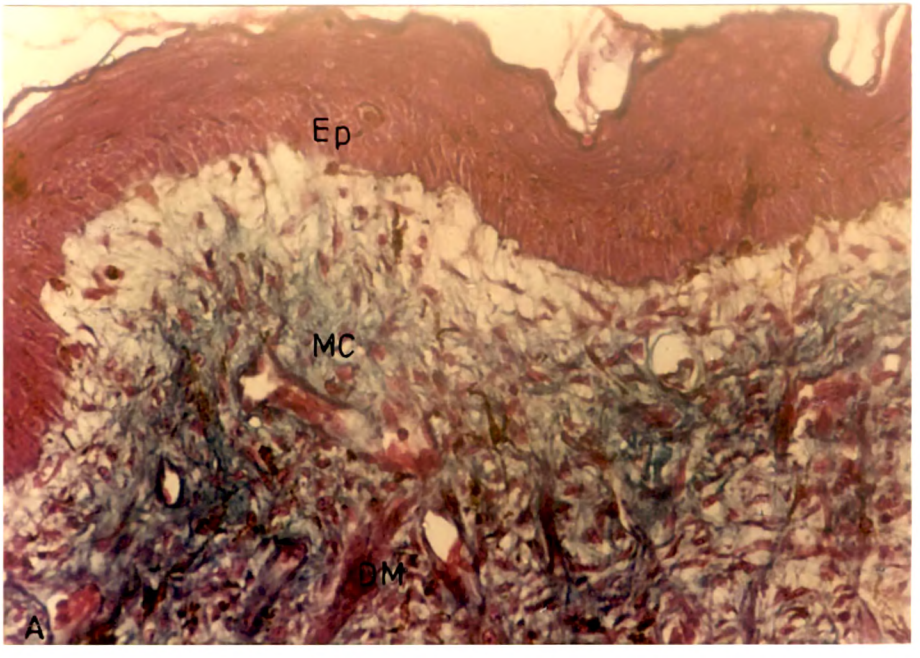
Photomicrographs of sections of regenerating tail at the arrested pre-blastema stage in EGF treated lizards.

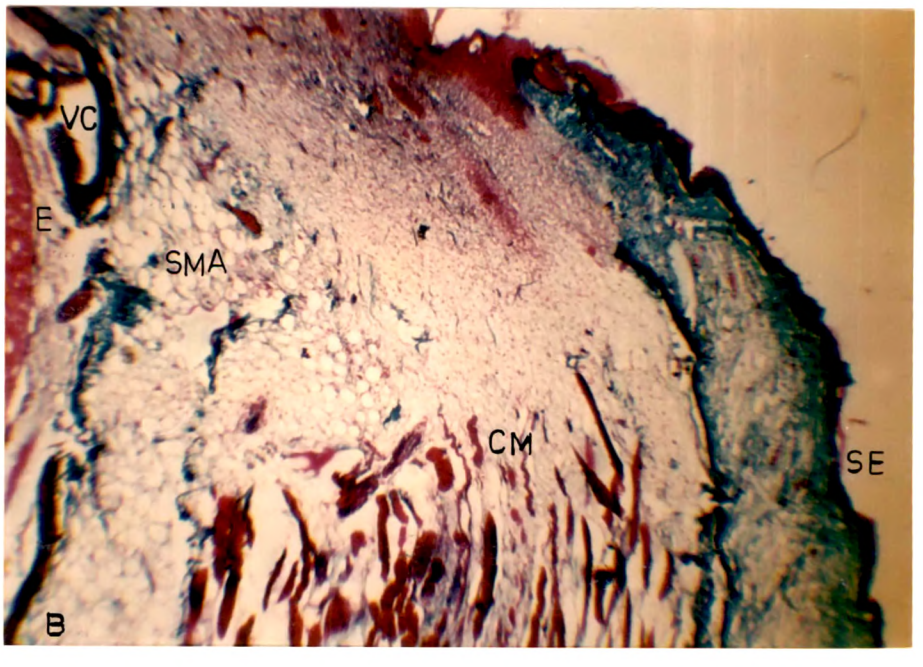
A - (300 X) B - (150 X)

A. Enlarged view of the tip of the pre-blastema showing presence of collagen (greenish-blue) below the thickened epithelium (Ep). Also seen are the mesenchymal cells (MC) and precociously differentiating muscle cells (DM).

B. Lateral half of the above tail section showing the presence of thick collagen (green) below the stump epidermis (SE). Also seen are the cut end of the muscles (CM), submuscular adipose tissue (SMA), vertebral column (VC) and part of ependyma (E).

PLATE - 8.7





LEGENDS TO FIGURES

Plate - 8.8

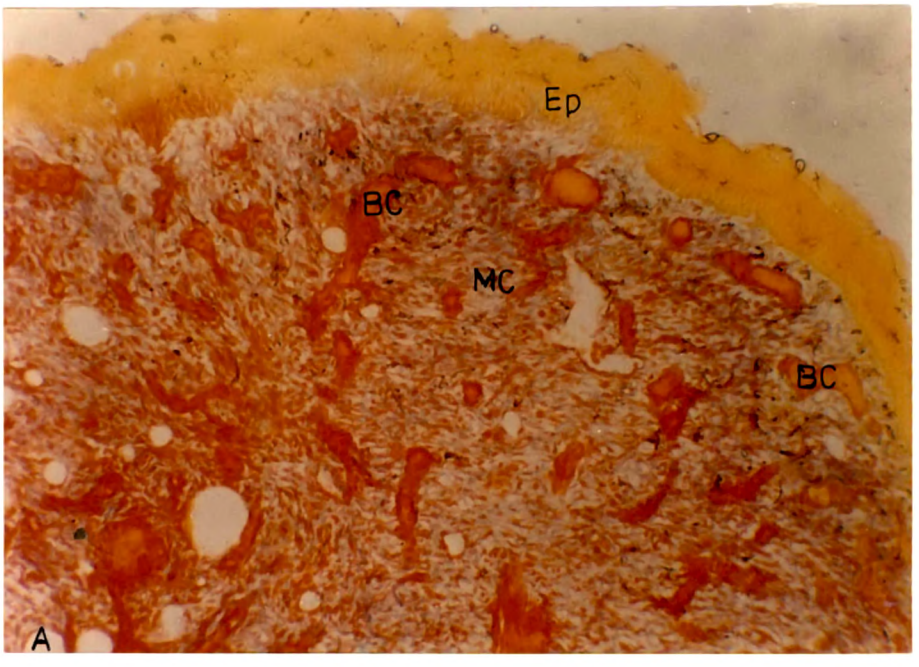
Photomicrographs of the sections of regenerating tail of lizard treated with EGF in the pre-blastema stage.

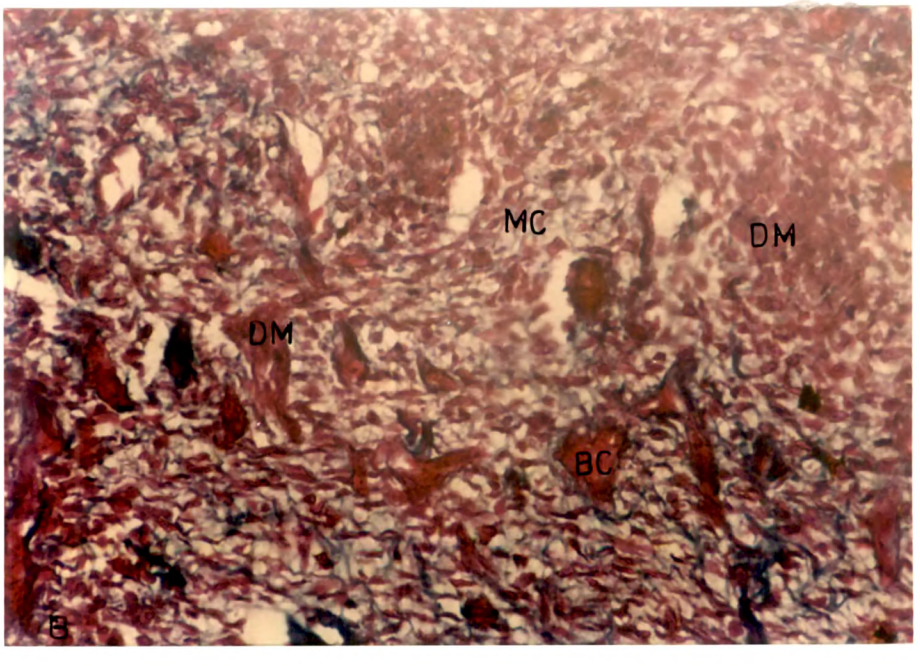
A - (150 X) B - (200 X)

A. The tip of the pre-blasstema showing thickened epithelium (Ep), mesenchymal cells (MC), blood cells (BC)- yellow and differentiating muscle tissue (DM) - orange. Casson's stain.

B. Enlarged version of the mesenchymal mass showing loose mesenchymal cells (MC), blood cells (BC) - red, differentiating muscle tissue (DM) - red and collagen material (green). Mallory's triple stain.

PLATE- 8.8



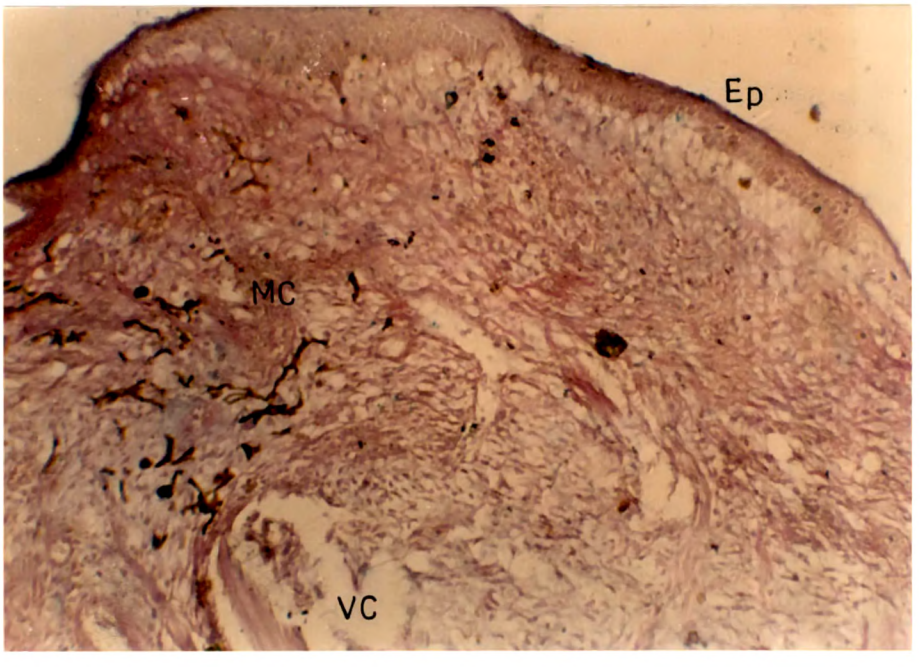


LEGEND TO FIGURE

Plate - 8.9

Photomicrograph of the section of a regenerating tail arrested at the pre-blastema stage from a lizard treated with EGF (150 X). PAS staining. Note the loose mesenchymal cells (MC), thick epithelium (Ep) and some GAG material (purplish in colour) only around the vertebral column of the tail stump but not in the differentiating cartilage area.

PLATE - 8.9



LEGENDS TO FIGURES

Plate - 8.10

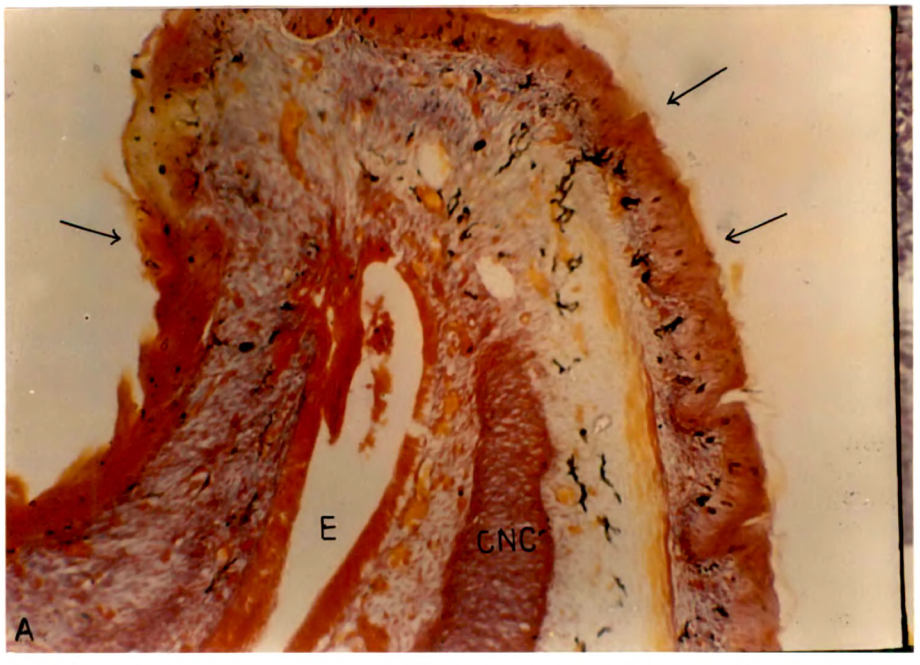
A & B: Photomicrographs of the sections of a regenerating tail with stunted hooked growth from a lizard treated with EGF.

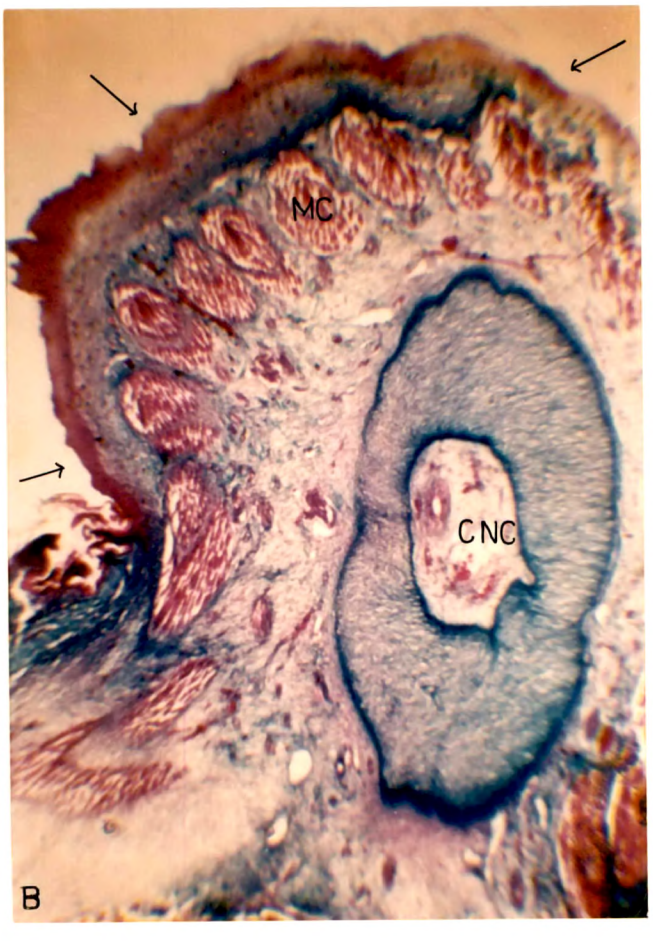
A - (150 X) B - (50 X)

A. Hooked tip of the regenerate showing precocious differentiation and keratinization of the epidermis (arrows). Also seen are the carilaginous neural canal (CNC) and ependyma (E). Casson's stain.

B. The tip of a hooked tail showing precocious epidermal differentiaion arrows and muscles (MC). Also seen are the cartilaginous neural canal (CNC) and a heavy deposition of collagen (greenish-blue) below the epidermis and around the muscle bundles as well as in the cartilaginous neural canal. Masson's trichome stain

PLATE ~ 8.10



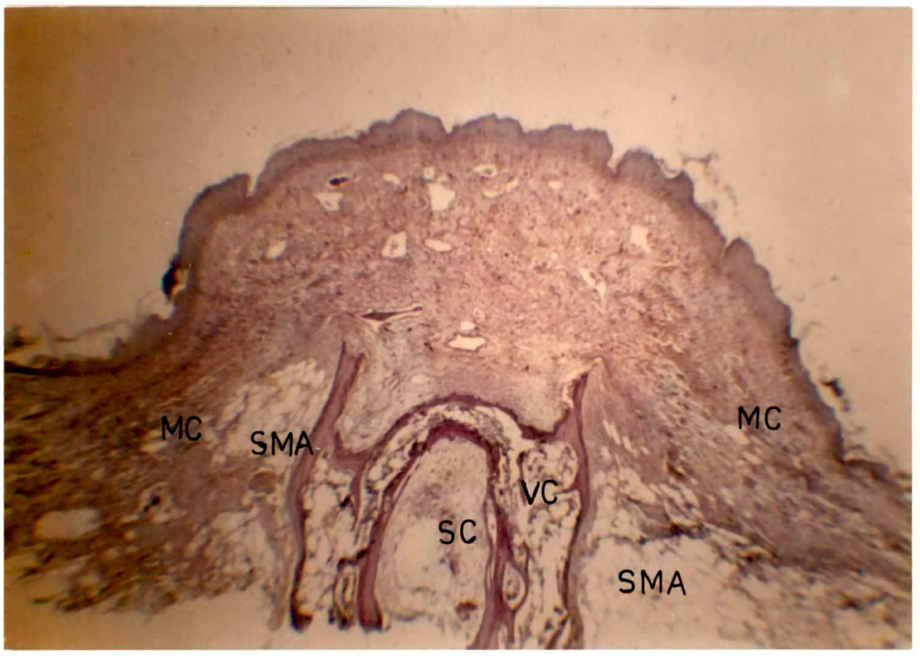


LEGEND TO FIGURE

Plate - 8.11

Photomicrograph of the section of a regenerating tail arrested at the preblastema stage from a lizard treated with TGF-β (50 X). PAS stain. Note the thickened epithelium with loose mesenchymal cells and presence of diffused GAG material (purple tinge). Also seen are the cut end of the vertebral column (VC) with spinal cord (SC), sub-muscular adipose tissue (SMA) and the muscle tissue (MC) in the stump area.

PLATE – 8.11



LEGENDS TO FIGURES

Plate - 8.12

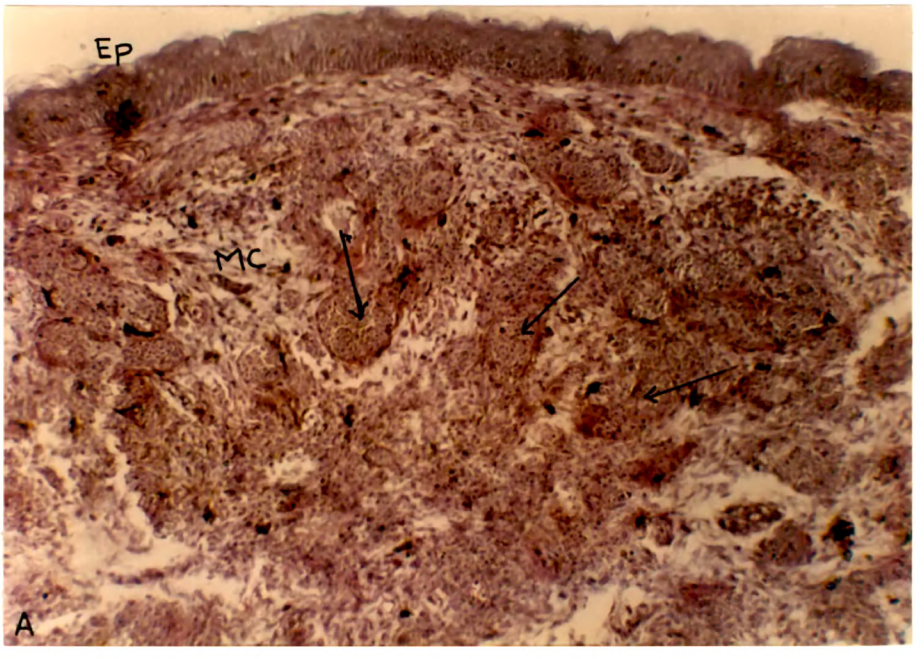
Photomicrographs of the section of regenerating tail arrested at the pre-blastema stage from a lizard treated with TGF - β

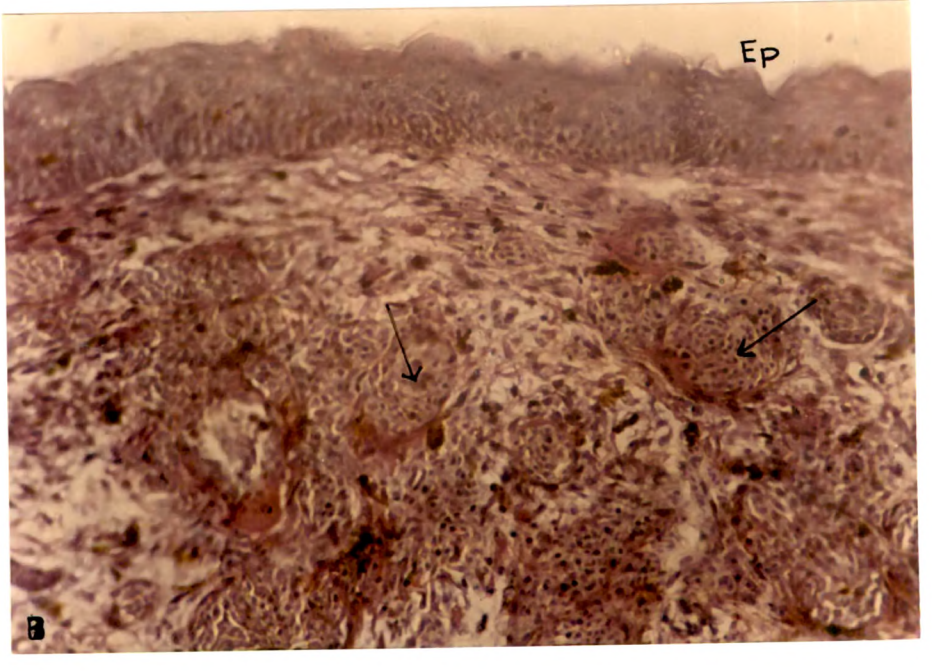
A - (150 X) B - (200 X) PAS stain.

A. The apical part of the pre-blastema showing the epithelium (Ep) and loose mesenchymal tissue (MC) packed with aggregations of RBCs (arrows).

B. Enlarged version of above, showing clearly the epithelium (Ep) and the RBC aggregations (arrows).

PLATE - 8.12





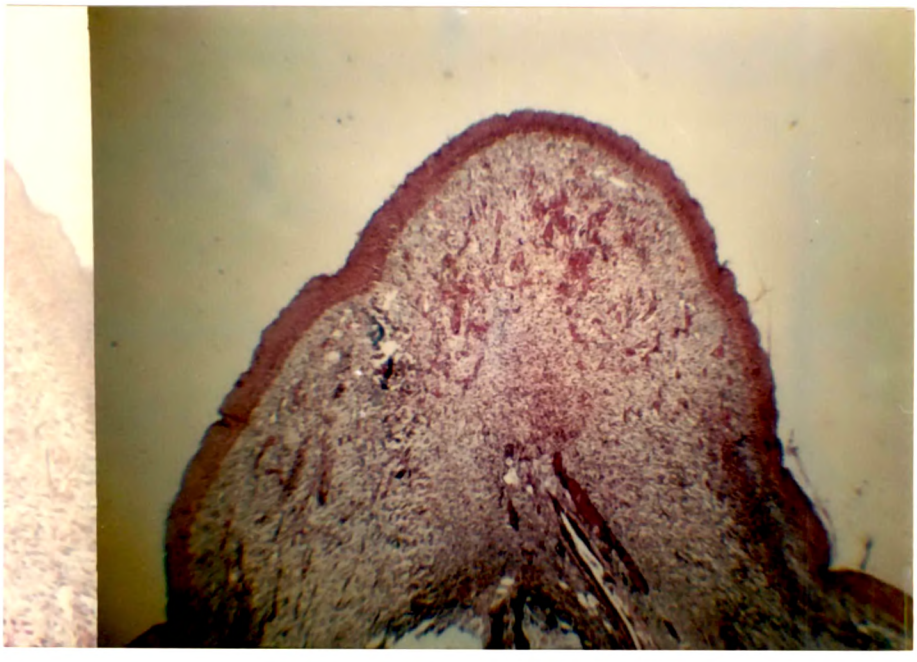
LEGENDS TO FIGURES

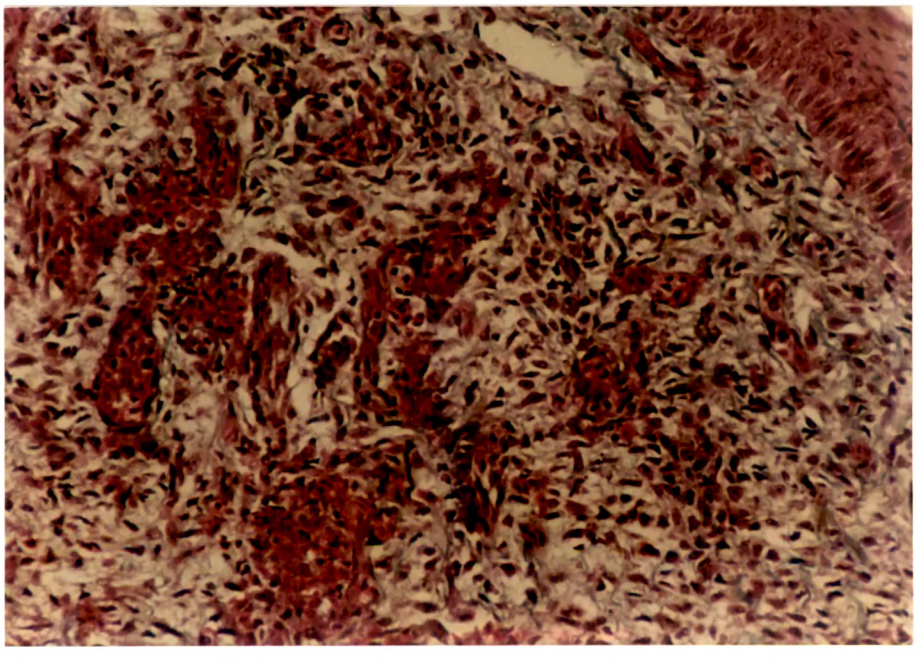
Plate - 8.13

A. Photomicrograph of the section of regeneration tail treated with TGF - β arrested at blastema stage (50 X) Mallory's Triple. Note the undedifferentiated muscle tissue (arrow) in the mesenchyme with no signs of differentiation Ep - Epithelium, MC - Mesechymal Cell, E - Ependyma, VC - Vertebral column.

B. Enlarged version of the same showing clumps of blood cells (BC) and undifferentiated muscle fibres.

PLATE - 8.13





LEGENDS TO FIGURES

Plate - 8.14

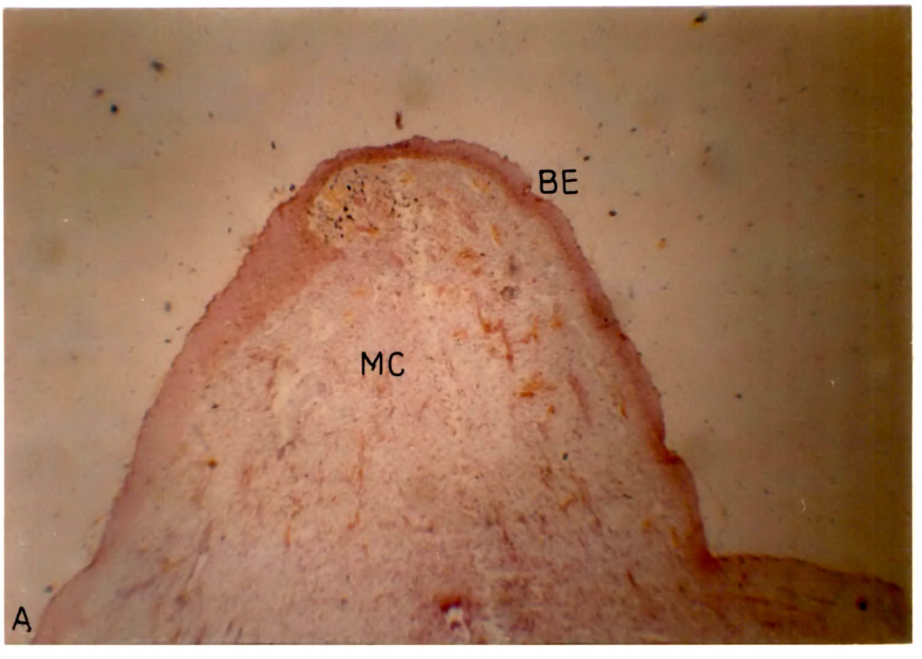
Photomicrographs of the sections of regenerating tail arrested at the blastema stage from a lizard treated with TGF - β

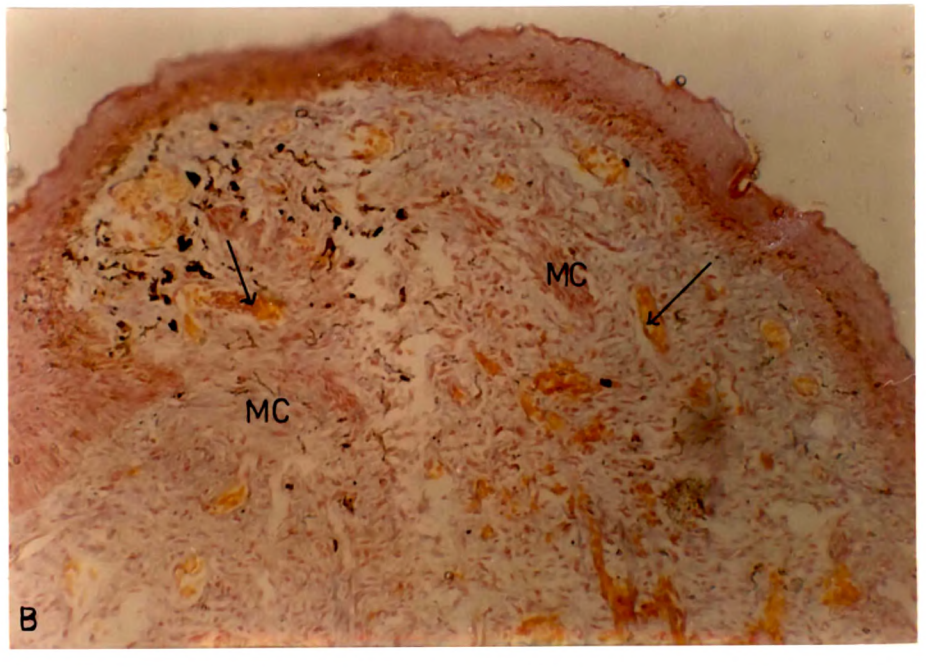
A - (50 X) B - (100 X) Casson's stain.

A. The appearance of blastema with no apparent signs of any differentiation. Blastemic epithelium (BE) and mesenchymal cells (MC).

B. An enlarged view of the tip of the blastema showing loose mesenchymal cells (MC) scattered accumulation of blood cells (arows, yellow colour), some undedifferentiated muscle fibres released from the cut end of the stump (MC) and cartilage condensation in the centre.

PLATE - 8.14





LEGENDS TO FIGURES

Plate - 8.15

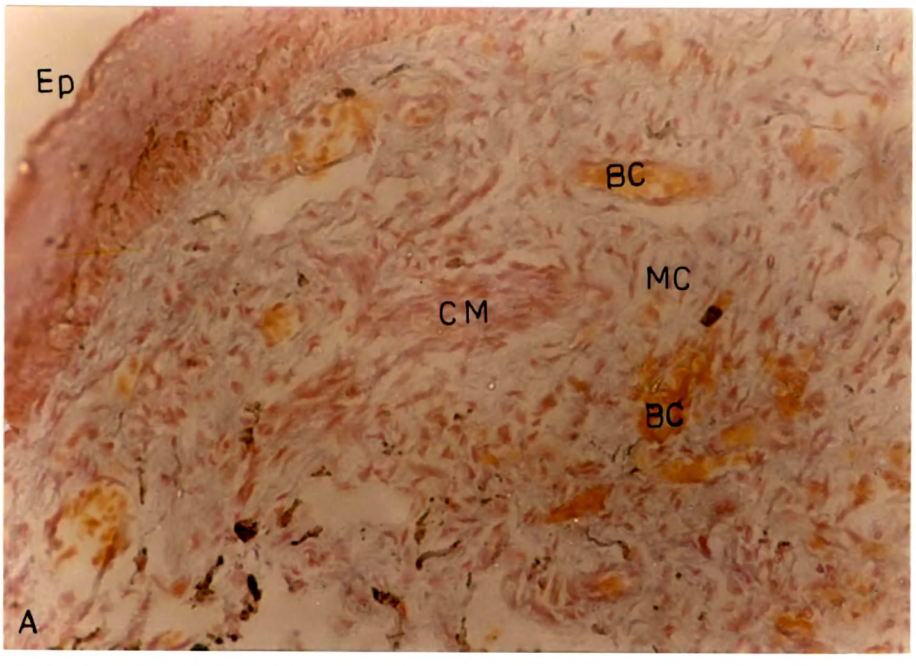
A & B: Photomicrographs of sections of the regenerating tails of a lizard treated with TGF- β arrested at an incipient blastema and pre-blastema stage. Casson's stain.

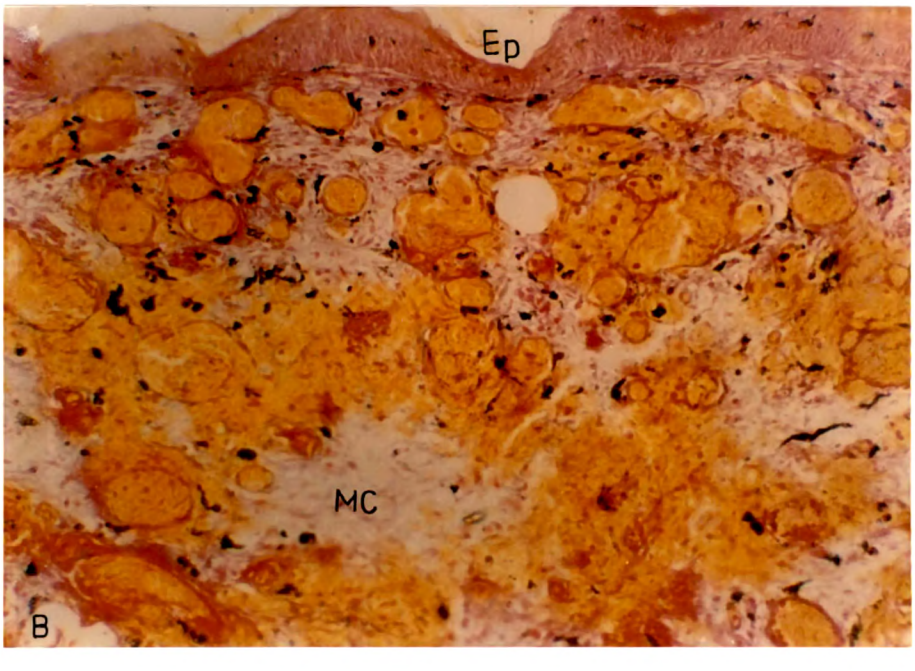
A - (100 X) B - (150 X)

A. An enlarged view of the tip of the incipient blastema showing epithelium (Ep), loose mesenchymal mass (MC), clumps of RBCs (BC) yellow colour and muscle fibres released from the cut end of the stump (CM)

B. The tip of the pre-blastema showing epithelial (Ep) and loose mesenchymal mass (MC) studded with clumps of erythrocytes (yellow colour).

PLATE - 8.15





LEGENDS TO FIGURES

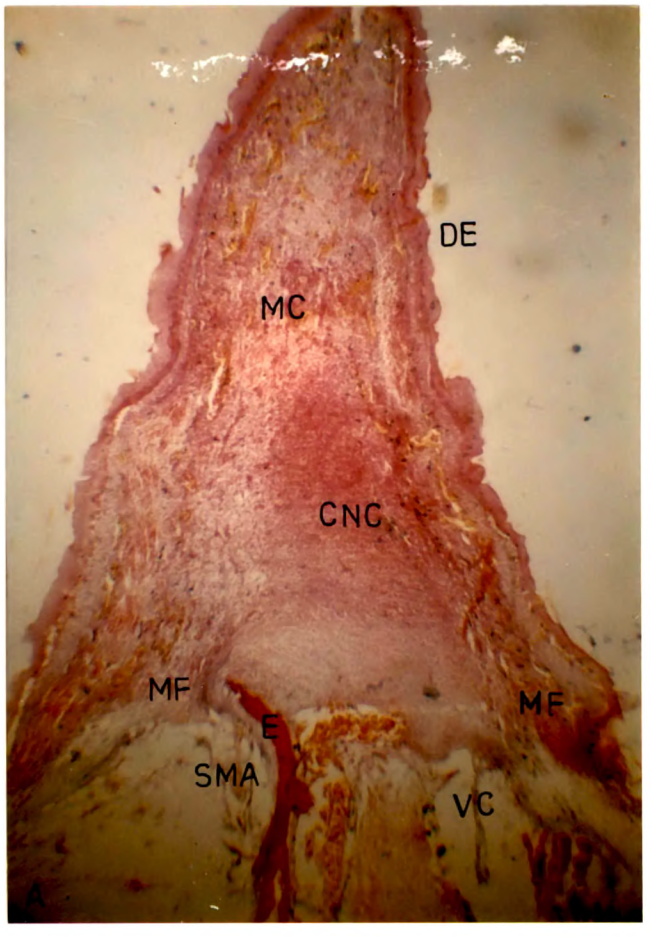
Plate - 8.16

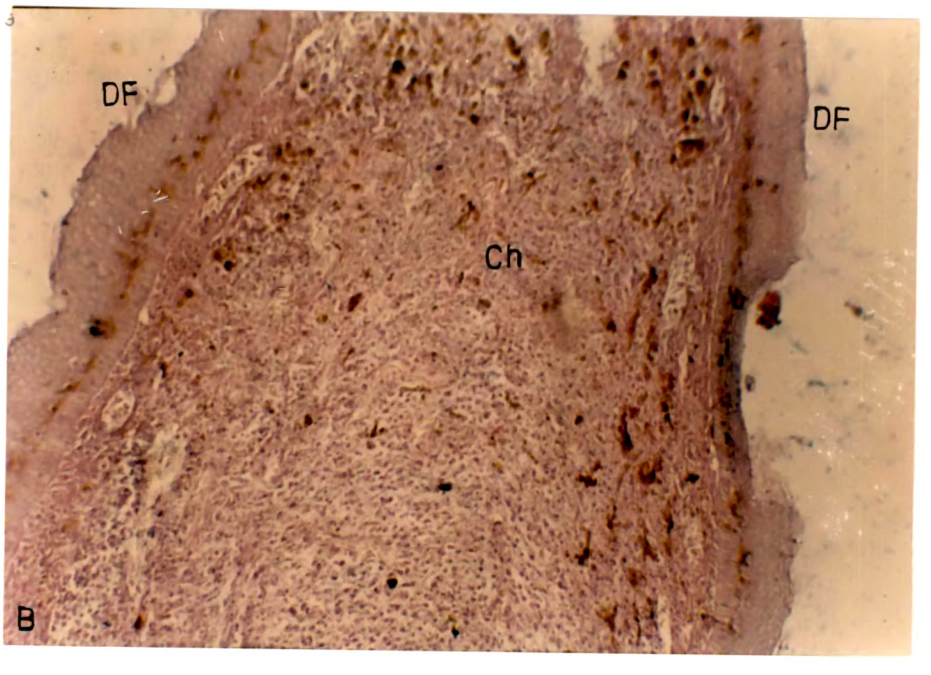
A & B : Photomicrographs of sections of regenerating tail of a lizard treated with TGF - β which produced a small regenerate after the cessation of TGF-β treatment A-(50X) Casson's stain β-(150X) PAS.

A : Showing the regenerate with no apparent signs of differentiation in the mesendymal tissue (MC) with only chondrogenic condensation. DE - Differentiating epithelium, arrow - scattered blood cells, CNC - cartilaginous neural canal, VC - Vertebral column, E - Ependyma, SMA - Submuscular adipose tissue, MF - Muscle Fibres.

B : Enlarged view of the above section showing only chondrogenic differentiation (Ch) in the mesenchymal tissue and GAG material (purple colour) DE - Differentiating epithelium.

PLATE - 8.16



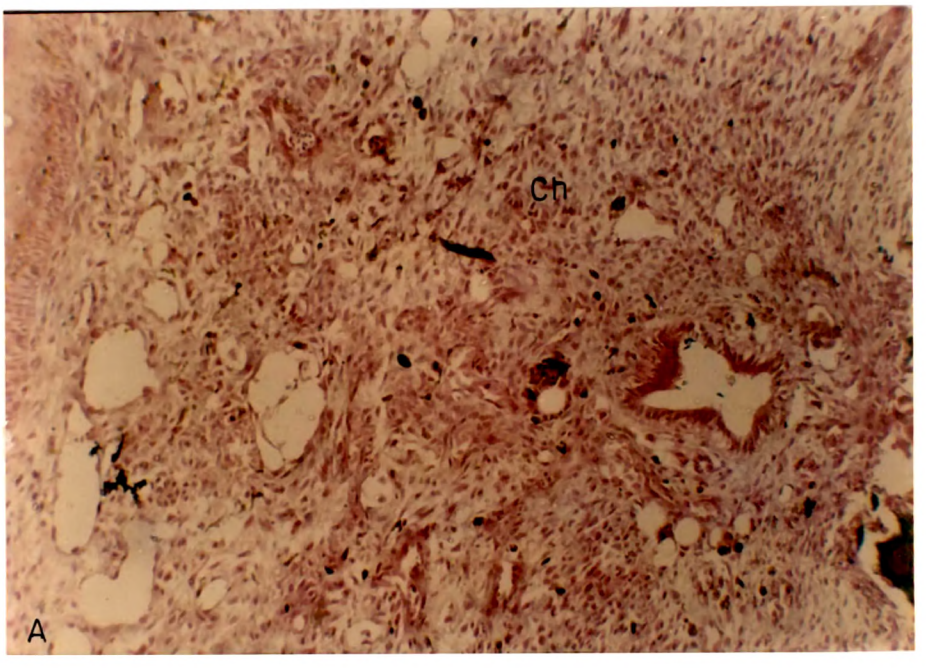


LEGENDS TO FIGURES

Plate - 8.17

A&B : Photomicrographs from the section of a regenerating tail of lizard treated with TGF-β A-(150X), B-(200X). PAS Staining. Showing extensive chondrogenesis with no other tissue differentiation. The purplish background in (B) represents GAG material.

PLATE - 8.17



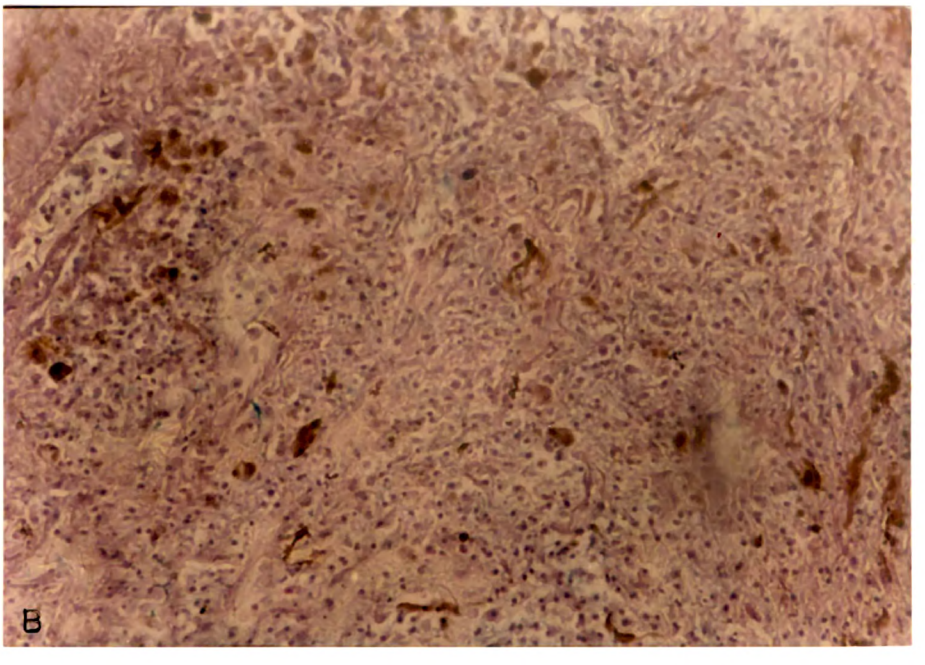
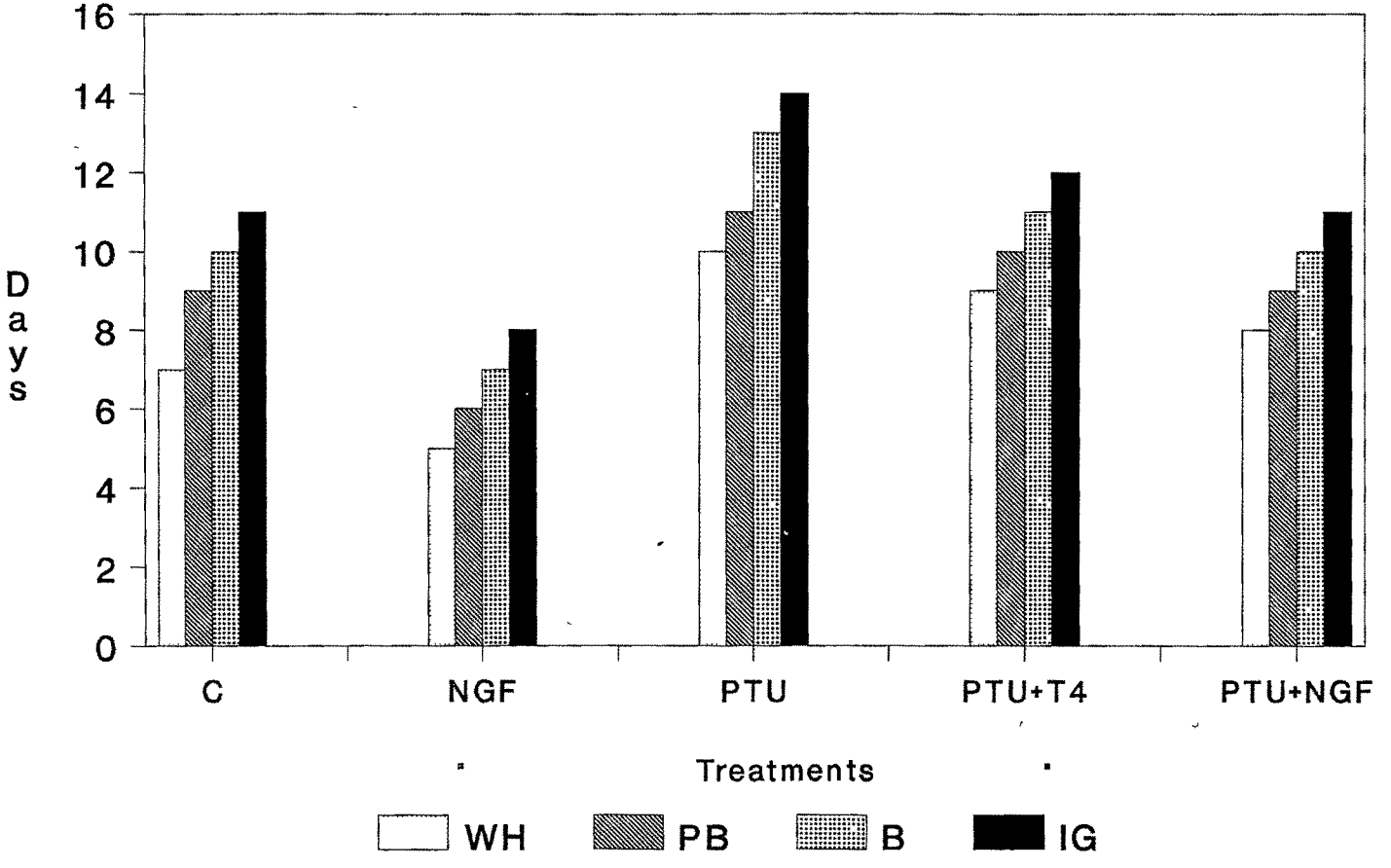
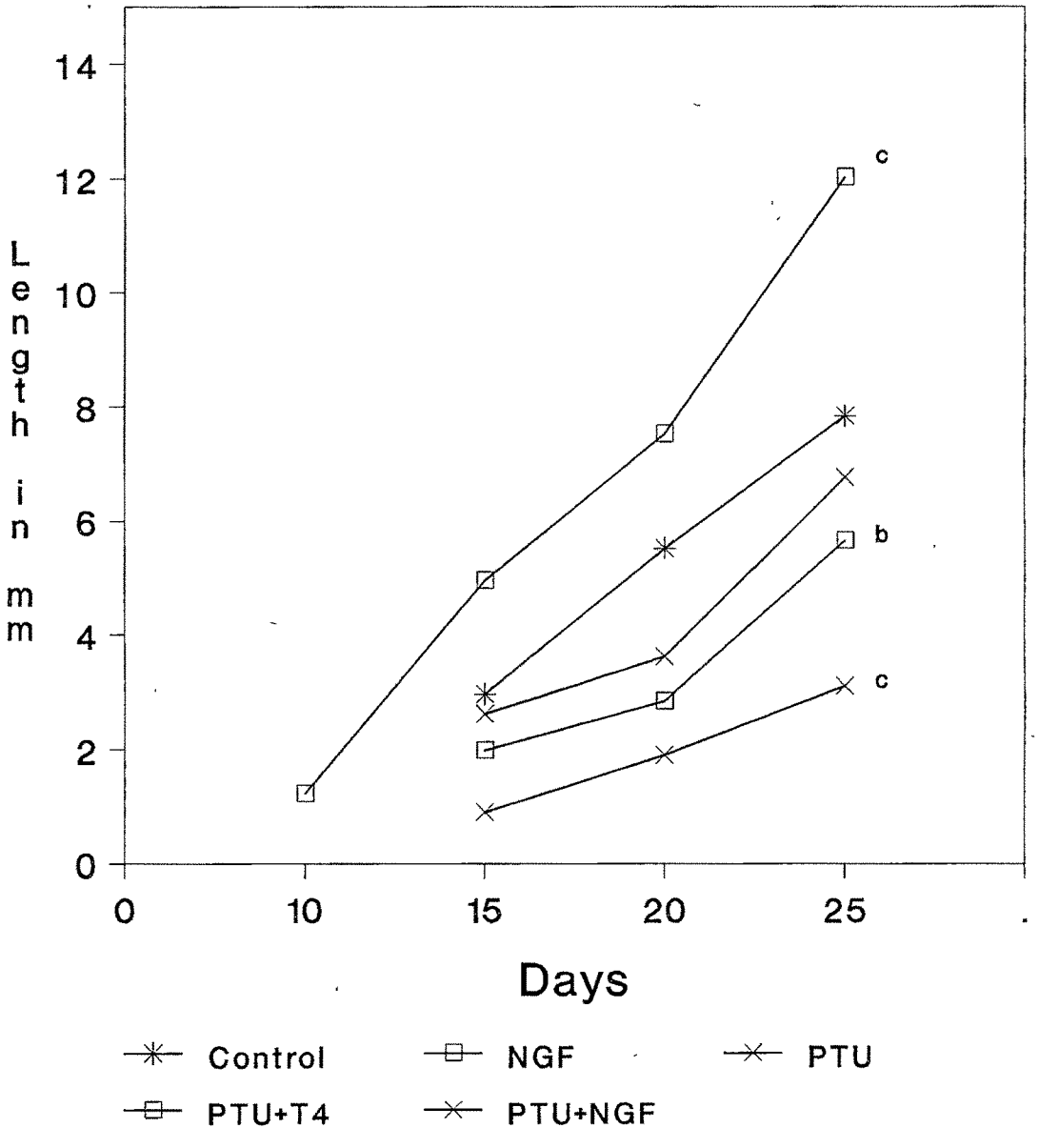


Fig.1 Number of days taken to attain the arbitrary stages in control (C) and experimental lizards.



NGF-Nerve Growth Factor PTU-Propyl thiouracil T4-Thyroxine

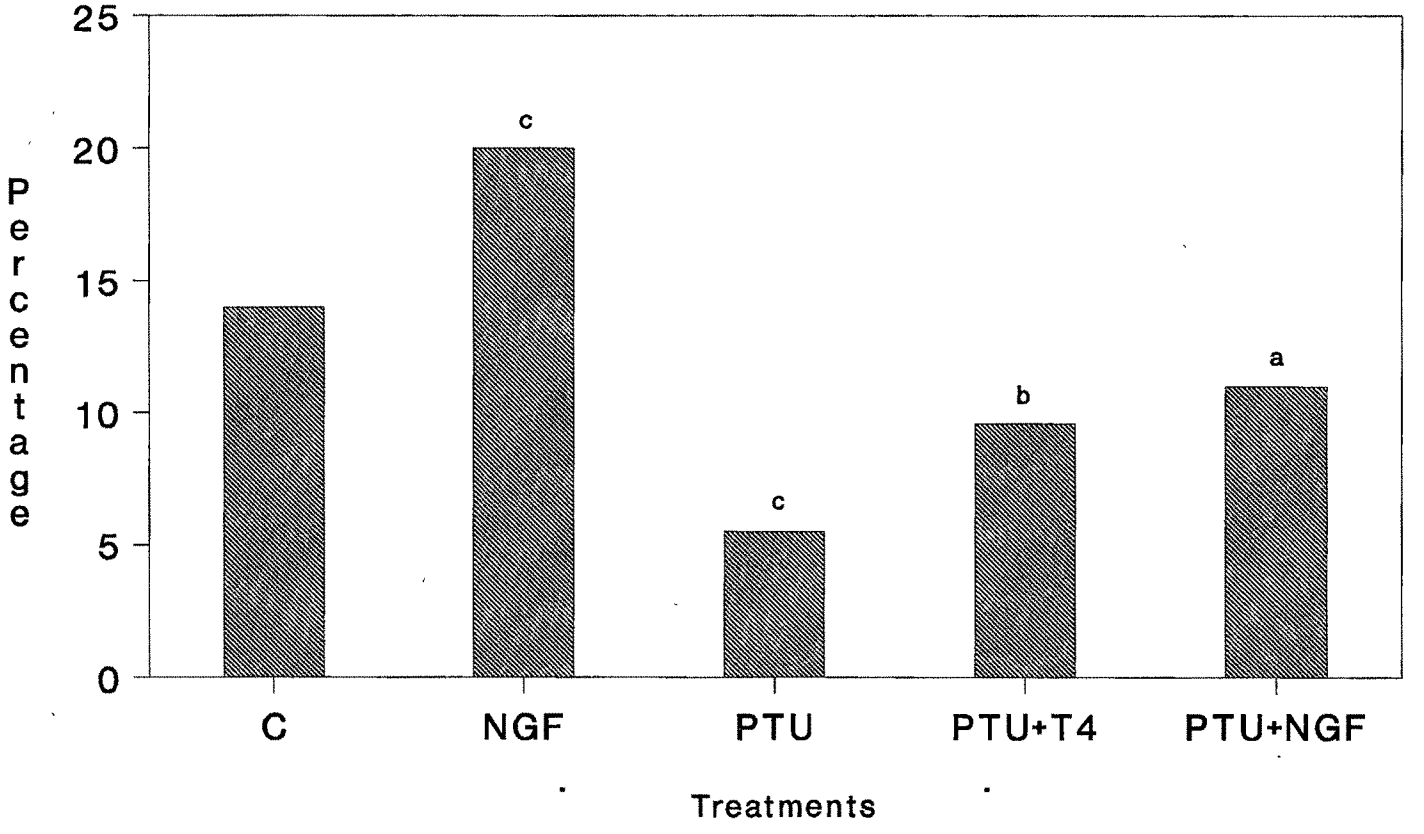
Fig.2 The length of tail regenerated in control(C) and experimental lizards at the end of 25 days.



NGF-Nerve growth factor PTU-Propyl-thiouracil T4-Thyroxine

b-P<0.005;c-P<0.001

Fig.3 The percentage of tail replaced in control(C) and experimental lizards.



T4-Thyroxine NGF-Nerve growth factor PTU-Propyl-thiouracil

a- P<0.01, b-P<0.005, c-P<0.001

TABLE - 3

Length of tail regenerated at different time period (days) at the end of 25 days in control and experimental lizards.

Manipulations	DAYS			
	10	15	20	25
CONTROL	-	2.96 ± 0.08	5.52 ± 0.26	7.84 ± 1.00
NGF	1.22 ± 0.36	4.96^{c} ± 0.75	7.52^{b} ± 0.70	12.02^{c} ± 0.93
PTU	-	0.90^{c} ± 0.09	1.9^{c} ± 0.17	3.1^{c} ± 0.21
PTU + T4	-	1.98^{a} ± 0.36	2.84^{c} ± 0.46	5.66^{b} ± 1.16
PTU + NGF	-	2.61 ± 0.34	3.62^{b} ± 0.45	6.76 ± 0.38

NGF - Nerve growth factor, PTU - 6-propyl thiouracil, T4 - T hyroxine.
a - $P < 0.01$, b - $P < 0.005$, c - $P < 0.001$.

TABLE - 4

Showing percentage replacement in control and experimental lizards at the end of 25 days.

Manipulations	Percentage replacement
CONTROL	14.00 ± 1.62
NGF	20.00 ± 2.28^{c}
PTU	5.5 ± 0.44^{c}
PTU + T4	9.6 ± 1.11^{b}
PTU + NGF	11.00 ± 0.98^{a}

NGF - Nerve growth factor, PTU - 6-propyl thiouracil, T4 - Thyroxine.
a - $P < 0.01$, b - $P < 0.005$, c - $P < 0.001$.

PTU Treatment : (Figs 1-3 and tables 2-4)

6-Propyl-thiouracil induced hypothyroidic lizards showed delayed wound healing, blastema formation and initiation of growth. The total length of tail regenerated was significantly less compared to the euthyroidic controls and the total percentage replacement at the end of 25 days was only 5%.

T4 and NGF Replacement : (Figs 1-3 and Tables 2-4)

All the retardory influence of hypothyroidism was completely nullified by either T4 or NGF replacement. Wound healing, blastema formation and initiation of growth occurred at time periods very much comparable with those noted for the control lizards. The total length of the tail regenerated and the total percentage replacement at the end of 25 days were much closer to those of the controls though slightly lesser on a comparative basis, NGF replacement seem to be a shade more effective than T4 replacement.

DISCUSSION

Of the three growth facotrs tested, EGF and TGF-β, showed inhibitory effects on regeneration while, NGF depicted significant favourable response. Given the tissue specific actions and differential effects on various cellular events and functions, the effect of growth factors on heteromorphic regeneration like the tail of a lizard is likely

to be complicated. The only growth factor which is studied and reported to have a stimulatory influence on lens and limb regeneration in amphibians is FGF (See Scheweigerer, 1990).

A purified preparation of FGF from the retina has been shown to stimulate lens regeneration in the newt (Cuny *et al.*, 1986). Similarly, FGF has also been shown to promote the formation of heteromorphic regenerating limbs in adult frogs as well as restore the regenerative capacity to denervated newt limbs (Gospodarowicz and Mescher, 1981). Apparently FGF is a strong evocator of wound healing and regeneration. However, orderly development and differentiation during vertebrate embryogenesis or even during appendage regeneration may depend on the action of various growth factors on a temporal and spatial plane. Be as it may, no other growth factor has been tested for its action/efficacy during vertebrate appendage regeneration. In this respect, the present study has revealed definite influence of three other growth factors in lizard tail regeneration.

EGF is a small single chain polypeptide of 53 amino acids first isolated from murine submaxillary glands by Cohen (1962). This polypeptide stimulates the proliferation of epidermal and epithelial cells in whole animals and a variety of cell types in culture (Carpenter and Cohen, 1979). Apart from promoting division of many tissues, including mammary epithelium and epidermis, it is necessary for the formation of the hard palate and the epithelial linings of the oesophagus, mouth and

cornea; it functions, both as a growth factor and as a differentiation-promoting hormone. When injected into new born mice, it accelerates the opening of the eyelids and the eruption of the teeth from the gum (Savage and Cohen, 1972). In keeping with its role in epithelial cell proliferation, in the present study, EGF treatment promoted early wound healing and formed a thickened multilayered blastemic epithelium. Though a delayed blastema with thickened epithelium was formed, its further outgrowth into a regenerate was either inhibited or stunted. A strong possibility for this effect on post-blastemic regenerative growth could be the heavy deposition of collagen below the epithelium (see plate 8.6). It is well established that during regenerative wound healing, the deposition of sub-epidermal collagen and/or scar tissue formation do not occur unlike in a non-regenerative wound healing (Schmidt, 1968; Shah *et al.*, 1980). Though the deposition of connective tissue material occurs on the lateral sides of the regenerate, it never occurs under the apical epithelium thereby permitting the continued elongation of the regenerate. As a consequence of the deposition of the dermal connective tissue on the lateral sides of the regenerate, the overlying epithelium undergoes extensive invaginations leading to scale formation and keratinization (see Alibardi, 1994). However due to the deposition of dermal substance all around the blastemic epithelium there was precocious and premature epithelial differentiation into scales and keratinization even at the apical region thereby inhibiting further regenerative growth. Another prominent influence of EGF in the present study was the precocious and preponderant myogenic differentiation.

The extensive myogenic influence resulted in increased muscle bundles with early differentiation into myotubes. This was evident even below the apical epithelium. This myogenic effect of EGF finds support from the observations of Lim and Hauschka (1984) and Olwin and Hauschka (1984) of proliferation of myoblasts in presence of EGF and the loss of receptors for EGF with the fusion of myoblasts and their differentiatioin into myotubes. Apparently, as in mammals, EGF is able to promote muscle differentiation in reptiles as well. Another observation of some merit is the less amount of glucosaminoglycan (GAG) material in the differentiating cartilaginous tube and the hooked tip of the regenerate, in those cases with stunted growth.

TGF-β is a member of a recently discovered family of polypeptides which seems to regulate cellular activity in organisms from drosophila to humans (Pfeilschifter, 1990). A very important action of this factor is on extracellular matrix synthesis and maintenance and, depending on the cell type it may exert stimulatory, inhibitory, biphasic or no effect on cell proliferation (Moses *et al.*, 1987).

Though TGF-β is capable of stimulating the growth of fibroblasts and osteoblasts, it inhibits the proliferation of many epithelial cells and has been shown to inhibit the growth of developing mammary gland (see Pfeilschifter, 1990). Another function accredited to this factor is in the various facets of wound healing, which has led to its being projected as a potential therapeutic agent for wound healing (Mustoe *et al.*, 1987; Pfeilschifter, 1990). However, this does not seem to be true for reptiles

as, in the present study, the post-caudal autotomy wound healing was not hastened in TGF-β treated lizards. In fact, the TGF-β treated lizards took the same number of days as the untreated lizards. The most conspicuous effect of TGF-β treatment in the present study was the formation of a poor regeneration blastema after a pronounced delay. Further, differential staining techniques revealed increased GAG deposition in mesenchymal mass. This observation is in agreement with the reported effect of TGF-β in stimulating the synthesis of all major matrix proteins, such as collagen and fibronectin (Ignotz and Massague, 1986), tenascin (Pearson *et al.*, 1988), elastin (Liu and Davidson, 1988) and glucosaminoglycan (Chen *et al.*, 1987) and thrombospondin (Penttinen *et al.*, 1988) The formation of a poor blastema as well as the inhibition in post-blastemic growth could be due to the inhibitory influence of this peptide on dedifferentiation of the stump tissues, as has been observed presently, and on proliferation of the dedifferentiated cells. The purported inhibition of the proliferation of the dedifferentiated cells is well neigh possible as TGF-β has been shown to be a potent inhibitor of growth of a wide variety of cells (Holley *et al.*, 1980; Proper *et al.*, 1982; Lawrence *et al.*, 1984; Roberts *et al.*, 1985; Pfeilschifter, 1990). A related observation is the inhibition of DNA synthesis in heptocytes under the influence of TGF-β in normal and regenerating liver of rat (Strain, 1990). Other observed anomalies consequent to TGF-β treatment are inhibited myogenesis and precocious chondrogenesis. These effects appear to be akin to what has been reported for

mammals, as TGF-β has been shown to stimulate chondrogenic differentiation and inhibit adipogenic and myogenic differentiation (see, Pfeilschifter, 1990). A novel feature observed in the present study with reference to TGF-β action is the stimulated haematopoietic activity, predominantly erythropoetic, in the adipose tissue of the tail stump. This has resulted in congregations of RBC's in the loose mesenchymal mass of the poorly formed blastema. In the wake of the hitherto unreported functional involvement of TGF-β in erythropoietic activity, the present observations implicate this growth factor in such a functional involvement in lower vertebrates. As against the inhibitory actions of the EGF and TGF-β, NGF has shown a definite stimulatory influence on regeneration in the present study. Local administration of NGF in the tail stump, post-caudal autotomy, significantly reduced the latent period (by 3 days) in blastema formation and provided greater momentum to growth in the initial phase. This is borne out by the significantly pronounced growth rate during the first 10 days post-autotomy.

Presumbly, the lizard tail has the potential to respond to exogenous NGF or a related peptide, and initiate regeneration. The observation of early wound closure in NGF treated lizards indicates its role in promoting wound healing. The report of Lui *et al.* (1980) had implied a role for NGF in wound healing. Besides, a possible role of NGF in altering cell properties which are more conducible for dedifferentiation and entry of cells into a proliferative state can also be pondered as, many reports suggest

pleiotypic responses like increased transport of nucleotides (Horii and Varon, 1977), glucose (Skaper and Varon, 1979a) and amino acids (Horri and Varon, 1977), synthesis of RNA (Levi-Montalceini and Angelatti, 1968a,b; Larrabee, 1969; Amaldi, 1971), lipids (Foppen *et al.*, 1969) and protein (Larrabee, 1969) besides, ionic transport, (Skaper and Varon, 1979b; Varon and Alder, 1980).

A probable clue to the source of NGF in the autotomized tail comes from the observation of the ability of exogenous NGF to nullify the delay in blastema formation exhibited by hypothyroidic lizards. Thyroxine has been implicated in the outgrowth of the ependyma "a priori" for regeneration to occur as it is responsible for providing the inductive influence for the initiation of regeneration in lizards (see Bellairs and Bryant, 1985). Since the delayed outgrowth of ependyma in hypothyroid lizards is responsible for retarded regenerative growth (Turner and Tipton, 1971) and in the present study, exogenous NGF could compensate for the lack of thyroxine and initiate a normal regenerative outgrowth, even better than that shown by T_4 replacement, it is conceivable that the ependyma could be a natural source of NGF in the lizard tail. Two relevant observations that lend credence to this assumption are, ultrastructural changes suggestive of secretory activity of ependymal tip (Turner and Singer, 1973; Alibardi and Sala, 1989) and the ability of thyroxine to increase the NGF content (Walker *et al.*, 1979). Another revealing evidence is the increased expression of NGF receptors subsequent to autotomy in the central nervous system

like, injury of the spinal cord (Brunello *et al.*, 1990) Though the ependyma has been recognized as the principal inducer of regeneration in lizards, generally ignoring the caudal nerves, it may not be wise to totally overlook the possible contribution of nerves. In fact Cox (1969b) had reported limited regenerative abilities of Anolis tail from which the spinal cord and the ependyma have been removed, provided 50-75% of the peripheral nerve fibres remain intact. Along with the partial regeneration of spinal cord after autotomy involving the ependyma, meninges, and glial cells, considerable number of descending nerve fibres also regenerate (see Bellairs and Bryant, 1985). It has also been shown by Simpson (1970) that the ability of Anolis to regenerate nerve fibres in the spinal cord is confined to the tail. Similarly, Terni (1922) had reported that sympathetic trunks have unconsidered roles as they regenerate on either side of the caudal artery, at the same time as, nerve fibres are growing back from the spinal cord. All the above observations show the ability of spinal cord and sympathetic nerves to regenerate after caudal autotomy. In this context, the present findings tend to underscore the possible influence of NGF elaborated by the ependyma in inducing neurite outgrowth and other normal functions associated with NGF involving accumulation of neurofilaments, formation of microtubules and axonogenesis (Walker *et al.*, 1979; Collins and Dowson, 1983). It is likely that NGF also induces the formation of the neurotropic factor which, or even NGF itself, induces mitotic activity in the apical epithelial cap. In conclusion, it may

be presumed that, the ependyma, under the influence of thyroxine produces NGF required for neurite outgrowth and the formation and action of the mitogenic factor(s) which would contribute to continued regenerative growth.

Overall, the present study has revealed a positive influence of NGF on tail regeneration and a negative effect of both EGF and TGF-β by their multiple cellular and subcellular effects.

SUMMARY

As growth factors exert control over various cellular events associated with growth and development and, as information regarding the influence of growth factors on regeneration is scant, presently the effect of exogenous *in loco* administration of EGF, TGF-β and NGF during the first few days post-autotomy on the course of tail regeneration in *H. flaviviridis* has been assessed. Further, the efficacy of NGF administration to rectify the retardatory influence of hypothyroidism has also been evaluated. The results show that, both EGF and TGF-β inhibited tail regeneration while, NGF stimulated the same. Histologically, the EGF treated lizards showed greater collagen formation and precocious epithelial and myogenic differentiation. TGF-β showed inhibited dedifferentiation and preponderant chondrogenic differentiation. In contrast, NGF treatment not only hastened, but even nullified the retardative influence of hypothyroidism on tail regeneration. Overall, the present results show differential effects of growth factors on various aspects of cell proliferation and differentiation suggesting, the need for the action of various growth factors on a precisely synchronized temporal and spatial order for, a normal regenerative growth.