CHAPTER 4

Role of epidermal growth factor in tail regeneration in *Hemidactylus flaviviridis*

INTRODUCTION

Epimorphic regeneration has been extensively studied in amphibians, especially newts and salamanders (Lo *et al.,* 1993, Brockes, 1994, Christensen and Tassava, 2000; Kumar *et al.,* 2000; Brockes and Kumar, 2002) The process is under influence of many factors *viz.* hormones, neural factors, growth factors etc. Recently the focus has been shifted to the role of growth factors in epimorphic regeneration. Many growth factors have been shown to influence the process, like VEGF, FGF-2, EGF, TGF, NGF etc. The role of FGF-2 in lizard tail regeneration has been discussed elsewhere (Chapters I, II and III) Further, few studies have demonstrated the role of epidermal growth factor (EGF) in epimorphic regeneration as well (Pilo and Suresh, 1994; Kurup, 1997) The process of epimorphic regeneration involves many events like healing of the wound, proliferation of the dedifferentiated cells which contribute to the blastema, and the redifferentiation of the pleuripotent blastemal cells to form the lost appendage Immediately after amputation of the appendage, the first process to occur is the healing of the amputated site, as discussed earlier. EGF is known to be involved in the healing cascade, which is a localized process involving inflammation, wound cell migration and mitosis, neovascularization, and regeneration of the extracellular matrix In addition, EGF is synthesized by several cells involved in wound healing including platelets, keratinocytes, and activated macrophages. Moreover, healing of a variety of wounds in animals and patients is enhanced by treatment with EGF (Schultz *et al.,* 1991). In addition, combined application of recombinant human EGF (rhEGF) with recombinant human FGF-2 (rhFGF-2), is found to be more beneficial to the wound repair (Xing *et al.,* 2004). Besides, EGF application to cultured mouse intestinal epithelial cells dramatically increases migration into a wounded area (Polk, 1999), and similar results have been reported with rabbit duodenal organ cultures (Riegler *et al* ,1996) as well as cultured human colonic cell lines or epithelia (Riegler *et al* ,1997,Wilson, and Gibson, 1999;Pouliot *et al.*, 2001). Furthermore a recent clinical study indicates that topical EGF application is effective in treatment of ulcerative colitis (Sinha *et al.*, 2003), suggesting that the promotion of wound healing by this factor *in vitro* effectively models *in vivo* responses

Apart from its role in wound healing, EGF is also known as a proliferation inducing factor (Carpenter and Cohen 1990, Broecker *et al*, 1998). EGF has been shown to be a mitogenic agent *in vitro* as well as *in vivo* For example, EGF stimulates mitogenesis of cultured neuroprogenitor cells (Ghosh *et al.,* 1995, Temple *et al*, 1995, Vicario-Abejón *et al.,* 1995) and also plays an important role in cell proliferation during embryogenesis (Iamaroon *et al*, 1996) The activity of epidermal growth factor and its receptor the EGFR have been identified as key drivers in the process of cell growth and replication (Goustin *et al*, 1986, Aaronson, 1991) Heightened activity at the EGF receptor, whether caused by an increase in the concentration of ligand around the cell, an increase in receptor numbers, a decrease in receptor turnover or receptor mutation can lead to an increase in the drive for the cell to replicate There is now a body of evidence to show that the EGFR-mediated drive is increased in a wide variety of solid tumors including non-small cell lung cancer, prostate cancer, breast cancer, gastric cancer, and tumors of the head and neck (Salomon *et al.*, 1995) Epidermal growth factor is thought to play a role in tumor development and metastasis EGF receptor overexpression has been detected in a variety of human breast cancer cells (Murphy *et al*, 1990) It has been shown to modulate the growth of mammary gland cells as well as the proliferation of cultured hormone-sensitive human breast cancer cells (Cupis and Favoni, 1997) EGF has potent growth-promoting effects in mammary epithelium, stimulating DNA synthesis and cellular proliferation (Murphy *et al*, 1996) Much evidence supports the view that peptide growth factor pathways are intimately involved in the proliferative response of breast cancer cells (Dickson and Lippman, 1987).

Moreover, it is well established that in order for a quiescent cell to proliferate, it must be continually exposed to mitogen until a few hours prior to S phase (Pardee, 1974, Jones, and Kazlauskas, 2001). In serum-arrested epithelial cells, as well as in many other cell types, this is typically a span of 7 to 9h, entailing the initial entry into early G_1 phase from the quiescent state (or G_0), and on through late G_1 and past the restriction point (R point) (Pardee, 1974; Pardee, 1989; Planas-Silva and Weinberg, 1997, Balciunaite et *al.,* 2000; Jones and Kazlauskas, 2001). Formation of the receptor-signaling protein complexes of EGF and EGFR, initiates the activation of various signaling pathways leading to cell proliferation (Carpenter, 1987, Schlessinger, and Ullrich, 1992, Pawson, 1995, Pawson, 1997, Yarden, and Sliwkowski, 2001). Furthermore, excessive activation of EGFR on the cancer cell surface is now known to be associated with advanced disease, the development of a metastatic phenotype Activation of EGFR by epidermal growth factor (EGF) and other ligands which bind to its extracellular domain is the first step in a series of complex signaling pathways which take the message to proliferate from the cell membrane to the genetic material deep within the cell nucleus Epidermal growth factor has an important role in maintaining cellular homeostasis due to its role in proliferation, apoptosis and differentiation

Binding of EGF to its receptors induces stimulation of its tyrosine kinase activity, phosphorylating multiple cellular substrates including itself, and eventually these events lead to cell proliferation (Ullrich A and Schlesinger, 1990) Stimulation of proliferation by EGF has been demonstrated in normal as well as cancer cells (Carpenter and Cohen, 1990, Goustin *et al,* 1986)

Thus, from the studies carried out by several investigators, it has been established that EGF is involved in the proliferative activities, most noticeably in several kinds of tumors Moreover, it is also involved in the epimorphic regeneration Hence, the present study was planned to evaluate the mitogenic potential of EGF and its influence on the regeneration of tail in the wall lizard, *Hemidactylus flaviviridis.*

MATERIALS AND METHODS

Adult wall lizards, *Hemidactylus flavivindis,* of both the sexes, with intact tail and weighing an average 10±2 gram, were selected They were acclimated for a week before experiments were started. The animals were fed with cockroaches as and when required (2 times a week) and water was given daily, *ad libitum.*

Experiment I:

Series A:

A total of twenty-four animals were used and they were divided into four groups of six animals each Animals in each group were treated as follows:

Group I. The animals of this group were treated with 0 6% saline intraperitoneally.
Group II Animals were injected intraperitoneally with Epidermal Growth Factor at a dosage of 25µg/kg b wt
Group III Lizards received *in loco* injection of physiological saline
Group IV This group of lizards received EGF *in loco,* at a dosage of 10µg/kg b wt.

The animals of this experiment were treated four days before autotomy was induced and the treatment was continued for six days

Series B:

A total of fifty lizards were amputated and the regenerating lizards were selected at two stages- (i) at the completion of wound healing and appearance of WE, and (ii) at the BL stage.

(i) WH stage. Twenty four lizards which attained WE stage on the same day were selected and divided into four groups of six animals each and were treated as follows-

Group I· They were injected with 0 6% saline, IP

Group II. Injected with EGF, IP (25 μg/kg b wt)

Group III. Saline was administered *in loco*

Group IV The animals of this group were administered EGF, *in loco* (10 μg/kg b wt.)

The drugs were prepared fresh in physiological saline The treatment was given every alternate day and was continued for ten days

(ii) BL stage All those lizards which attained BL stage on the same day were divided into four groups and were treated in a manner similar to (i)

For treatments of both the series, the number of days taken by the animals to reach various stages of regeneration and the length of the regenerate was recorded at fixed time intervals

Experiment II:

As many as eighty lizards were procured from the animal dealer and were kept in cages in the animal house, maintained at a temperature of 30 ± 2^0 C with 12 12 light dark cycles The animals were acclimated for a week before experiments. They were divided into four groups of twenty animals each and were treated as shown below:

Group I: The lizards of this group were injected intraperitoneally with physiological saline

Group II. EGF was administered intraperitoneally to lizards at a dose of 25 μg/kg b wt

Group III: Animals were injected with saline *in loco.*

Group IV: *In loco* injection of EGF was given to animals at a dosage of 10μg/kg b.wt

The treatment was started four days prior to autotomy and was continued till the animals attained DF stage. The lizards were sacrificed at two stages *viz.* BL and DF stages and the nucleic acids and protein levels were determined in the regenerates.

Histofluorescent localization of nucleic acids: The regenerates from the above groups were cut and transferred to cryostat microtome maintained at –25^{0}C Tissues were fixed in Tissue Tek-II and sectioned at 10μm. The sections were stained with acridine orange and observed for nucleic acids localization under fluorescence microscope (DMRB, Leica) with epi-illumination with filter settings of 440 nm excitation filter and 510 nm barrier filter.

STATISTICAL ANALYSIS: The data on the number of days taken and the growth rate was analyzed using Student's 't' test and is expressed as Mean ± SE. Other tests were performed by One Way ANOVA with Duncan's Multiple Range Test. A p value of 0 05 or less was considered statistically significant

RESULTS

Experiment I:

Administration of EGF Prior to autotomy: Administration of EGF prior to autotomy to the lizard, *Hemidactylus flaviviridis*, was found to enhance the tail regeneration In the EGF treated lizards, both IP and *in loco* treatments, the wound was healed four days prior as compared to control lizards (Table 4.1a) The blastema formation was slower in control lizards as compared with treated animals. Further, measurement of length of the regenerate revealed a boost in the growth during early events of tail regeneration in experimental animals The rate of growth, from 2-12 mm was significantly higher ($p \leq 0.05$) in the regenerates of the EGF treated animals (Figure 4.1a) There was approximately 78% and 73% increase in the rate of growth of regenerate from 2-12 mm, in the EGF treated lizards, IP and *in loco* treatments, respectively, while from 12-24 mm there was 21% and 33% increase respectively The rate of growth was significantly higher in the EGF treated lizards from 12-24 mm, both IP ($p \leq 0.05$) and *in loco* ($p \leq 0$ 01) (Figure 4.2a) The differentiation in regenerates of the EGF treated animals started earlier than the control animals.

Administration at WE stage: The animals treated with EGF at WE stage developed blastema faster than control animals (Table 4 1b) There was a significant ($p \leq 0$ 01) acceleration in the proliferative activities in the regenerate of EGF treated animals as compared to control animals The rate of growth of regenerate from 2-12 mm was found to be significantly higher ($p \leq 0$ 05) in treated animals (Figure 4 1b), while there was 36% and 31% increase in the length of regenerate in IP and IL treated animals respectively. Further, the rate of growth from 12-24 mm was found to be significantly higher in the IP ($p \leq 0$ 05) and

IL ($p \leq 0.01$) treatments as compared to control animals (Figure 4.2b), with 16% and 24% increase in the length of regenerate respectively. Moreover, the initiation of differentiation was found to be comparatively enhanced in EGF treated animals when compared with control animals.

Administration at BL stage: Treatment with EGF at BL stage increased the rate of growth of regenerate during initial events, but later on, there was not significant increase in the growth rate of regenerate as compared to control animals (Table 4.1c). There was not any significant change in the rate of growth of regenerate form 2-12 mm or 12-24 m, except in the IL treatment where there was a significant increase ($p \leq 0.01$) in the growth rate from 2-12 mm only (Figure 4.1c and 4.2c). Though initially there was approximately 23% and 17% increase in the length of regenerate from 2-12 mm growth, but during 12-24 mm growth of the regenerate the percentage increase dropped down to 3% and 5% in the IP and IL treatments respectively.

Experiment II:

BL Stage: The DNA levels of the EGF treated lizards were significantly higher ($p \leq 0.01$) in the regenerate, at the BL stage as compared to control animals (Table 4.2; Figures 4.3, 4.13 and 4.14). Similarly, the RNA and protein levels were also significantly higher ($p \leq 0.01$) in the experimental lizards as compared to control lizards (Figure 4.5 and 4.7). Moreover, the DNA/RNA ratio was found to be appreciably lower ($p \leq 0.01$) in EGF treated lizards as compared to control animals (Figure 4.9). Similarly, the RNA/Protein ratio was found to be notably lower ($p \leq 0.01$) in the treated animals as well (Figure 4.11).

DF stage: At differentiation stage, a significant hike ($p \leq 0.01$) was observed in the DNA levels in EGF treated animals as compared to control lizards (Table 4.3; Figures 4.4 and 4.15). Though, RNA levels were considerably elevated ($p \leq 0.05$) in the experimental animals, the protein levels were not significantly different from the control animals (Figure 4.6 and 4.8). Further, the DNA/RNA ratio as well as RNA/protein ratio was comparable between the control and experimental animals (Figure 4.10, 4.12).

DISCUSSION

The present study revealed a positive influence of EGF on the tail regeneration in *Hemidactylus flaviviridis*. The administration of epidermal growth factor to the animals

accelerated the process of wound healing after autotomy of the tail The faster healing of the wound might be due to the rapid closure of the wound by the epithelial cells Thus, EGF might be stimulating epithelial cell proliferation during healing process and the proliferation of epithelial cells of the amputated site might be accompanied by the migration over the wound surface. Davies *et al.,* (1999) have shown that activation of the epidermal growth factor receptor (EGFR) following ligand binding may play an important role in epithelial repair processes by inducing cell migration, proliferation, and differentiation in humans. Moreover, Xu *et al.,* (2004) have shown that epithelial wound healing is, at least in part, mediated in an autocrine fashion by epidermal growth factor receptor (EGFR)–ligand interactions in human corneal epithelial cells Studies have also shown that EGFR is activated during corneal epithelial wound healing *in vivo* Furthermore, this activation appears to be a necessary component of the process, because inhibition of the EGFR signaling cascade significantly slows migration rates during healing of the wound (Zieske *et al*, 2000) Besides, epidermal growth factor participates in wound healing by increasing reepithelization and reparation tissue strength via enhancing collagen deposition to the wound site in mice (Babul *et al*, 2004) Similarly, EGFR signaling has been implicated as a potent inducer of intestinal cell migration and wound healing in rats (Blay, and Brown 1985; Wilson, and Gibson, 1997; Egan *et al.,* 2003) and in human corneas, EGF is found promoting endothelial wound healing predominantly by cell migration as reported by Hoppenreijs *et al*, (1992) Thus, from all these studies it is logical to surmise that in the healing of the wound during lizard tail regeneration, EGF might be stimulating the proliferation and migration of epithelial cells over the amputated surface and thus could bring about early healing of the wound and the formation of WE

The healing of the wound during tail regeneration was followed by the proliferative activities of the pleuripotent cells that added to the pool of blastemal cells In the current study, the animals treated with EGF showed a heightened proliferation of cells and thus the regenerate showed a higher rate of growth as compared to control animals Also, *in vitro* studies have shown that EGF has the capacity to trigger cell proliferation in the development of the epithelial-mesenchymal organs of the mouse (Partanen, 1990, Weiss *et al.*, 1996) and this will also help the cells rescue from apoptosis (Leu *et al.*, 2000). Though, EGF has been shown to be mitogenic for neurons (Reynolds and Weiss, 1992; Kilpatrick and Bartlett, 1993, Ray *et al.,* 1993), in the regenerating blastema in lizards, it might be promoting proliferation of the blastemal cells, which are pleuripotent in nature The observed accelerated growth of the blastema in EGF treated animals could be attributed to the influence of extraneous EGF. Hence, the lizards treated with EGF showed an early attainment of BL and DF stages Further, Geimer and Bade (1991) have demonstrated that EGF is a potent mitogen for most cultured cells and has previously been shown to induce the migration of rat liver epithelial

cells Thus, the proliferative role of EGF might have been brought about by binding with its receptors Further, EGF binding to the EGFR might have initiated a number of signal transduction pathways, including Ras/Raf/MEK/ERK, phospholipase C-γ/PKC, and PI 3-kinase/Akt that are known to regulate cellular functions in mouse intestinal epithelial cells as opined by Kaiser *et al.,* (1999). The interactions between the proteins in the activated receptor complex are known to trigger the next step in the signaling pathway - the activation of a protein called ras which, in turn, initiates a cascade of phosphorylations which activate mitogen activated protein kinase (MAPK) MAP kinase takes the signal through the cytoplasm to the nucleus where it triggers events, which drive resting cells into cell division (Wells, 1999). Thus, the observed increase in the proliferative activities in the regenerate in treated animals could have been brought about by the above mentioned pathway. Moreover, studies have demonstrated the involvement of the EGF receptor and the MAP kinase signaling pathway in epithelial cell proliferation *in vitro* and *in vivo* (Wells, 1999, Andl *et al.,* 2003).

Furthermore, the rapid growth in the regenerate of the EGF treated animals might be due to increased proliferation of cells during early stages of tail regeneration. However, it could be argued that EGF might be pushing the adult quiescent cells of the amputated site into mitotic cycles Thus, such activated cells might have entered into proliferative cycles and contributed to the regeneration of the tail Further, the addition of exogenous growth factors such as epidermal growth factor is known to stimulate G_1-arrested FAO cells to enter the cell cycle (Chevalier and Roberts, 1999) Besides, the involvement of EGF in cell proliferation was further evaluated in the present study by the nucleic acids quantification as well as histoflourescence studies There was a significant increase in the DNA levels in the regenerate at the BL and DF stages in the lizards treated with EGF This observation was supported by the histofluorescence studies where a definite increase in DNA level was evident from the intense yellow fluorescence in the regenerates of the EGF treated animals. This indicated that there might have been an increase in the synthesis of DNA in the regenerates of the EGF treated Lizards. Moreover, Hyldahl (1986) has shown that EGF stimulates the initiation of DNA synthesis in the corneal endothelial cells *in vitro*, while Reddan and Wilson-Dziedzic (1983) reported that EGF increases cell division in lenses in culture. In addition, EGF accelerates DNA-synthesis in human corneas, although only to a limited extent (Hoppenreijs *et al.,* 1992). EGF at concentrations of 10^{-9} to 10^{-10} M initiates cell division in both confluent and low density non-dividing 3T3 cells (Rose *et al.,* 1975). However, addition of EGF either from mouse submaxillary gland or the human recombinant protein, appreciably stimulates thymidine incorporation and cell division approximately threefold (Kitchens *et al.,* 1994) Thus, the observed rise in the levels of DNA in the EGF treated animals was reflected in the high rate of proliferative activities induced by EGF

Moreover, along with its role in cell proliferation and growth of regenerate, EGF could also be involved in the synthetic activities during tail regeneration. The regenerates of the lizards treated with EGF showed manifold increase in the RNA levels during BL stage, while at DF stage there was a modest increase in treated animals as compared to controls Further, the DNA/RNA ratio was significantly lower in the experimental animals during BL stage while this was not statistically significant during DF stage These results indicated that EGF might be increasing the process of transcription in the early events of tail regeneration as was evident from the lower DNA/RNA ratio, but during DF stage the process was not influenced much in the experimental animals Moreover, Heath *et al.,* (1995) have stated that EGF increases the mRNA transcription in human keratinocyte cell line, while and Liu and Neufeld (2004) have reported the same in rat astrocytes Likewise, Lin *et al*, (2001) have also reported that EGFR complex participates directly in the transcription activation of cyclin D1, a key regulator of cell cycle progression in breast cancer cells Besides, inhibition of EGFR function strongly attenuated the global stimulation of protein synthesis by GPCR agonists *in vitro* in cultured aortic smooth muscle cells and *in vivo* in the rat aorta and in small resistance arteries (Voisin *et al.,* 2002). Histoflourescence studies also showed a high intense colouration for RNA in the regenerates at the BL stage Further, the RNA was very prominently localized in the lower margin of the apical epithelial cap (AEC), indicating that as the proximal cells of the AEC are engaged in proliferative activities, the distal ones start the process of transcription. However, in the proximal area of the regenerates there was found a region of active synthetic activity, which showed a high level of transcription activities Furthermore, the synthesized RNA needs to be translated into proteins to meet the demands of the rapidly growing regenerate The current study showed that the levels of proteins were increased at the BL stage in the EGF treated animals while remained similar to that of control animals at DF stage However, the RNA/Protein ratio was lowered in the regenerates of the treated animals at the BL stage, whilst this ratio was similar to controls at the DF stage. Thus, it could be postulated that EGF might be increasing the translational activities during initial events of regeneration of the tail, while after the differentiation starts the translational activities in the treated animals were found to be parallel with the controls. Moreover, that EGF enhances the translational activities, has been shown by many *in vitro* studies (Reynolds and Weiss, 1992, Vescovi *et al.,* 1993). Thus it can be argued that EGF could be enhancing the synthetic activities in the regenerate at the initial stages during the process of tail regeneration in the gekkonid lizard.

In summarizing the findings/observations, it could be hypothesized that the administration of extraneous EGF to the animals enhanced the process of tail regeneration in *Hemidactylus flavivirdis*, during early stages of regeneration There was a positive correlation between

EGF and the DNA levels in the regenerates of lizards. Similarly, the RNA and protein levels were also elevated in the treated animals. In addition, the EGF administration shortened the time taken by the animals for attaining WE and BL stage, but had little influence on the differentiating tail. Thus, EGF, as that of FGF-2, might be involved in the early events of tail regeneration. However, further study in this aspect needs to be carried out to gain in depth understanding of the mechanisms by which epidermal growth factor influences the regeneration of tail in *Hemidactylus flaviviridis*.

TABLE 4 1 Number of days taken by the animals to attain various stages of tail regeneration and the rate of growth in the treated and control lizards in different stage specific treatments *viz.* before amputation (Table 4 1 a), at WE stage (Table 4 1 b) and at BL stage (Table 4 1 c)

TABLE 4.1 a:

Treatment	Number of days		
	WH	BL (2 mm)	DF (12 mm)
IP Control	7 (6-7)#	10 (9-11)	17 (17-18)
IP EGF	3 (3-4)	6 (5-6)	13 (13-14)
IL Control	7 (7-8)	9 (9-10)	17 (16-18)
IL EGF	4 (3-4)	6 (5-6)	14 (14-15)

Treatment	Rate of growth		% increase/decrease compared to control $	
	From 2-12 mm	From 12-24 mm	From 2-12 mm	From 12-24 mm
IP Control	1.439±0 0583@	0 953±0 017	-	-
IP EGF	2 566±0 192*↑	1 156±0 024*↑	78↑	21↑
IL Control	1.582±0 044	0.927±0.020	-	-
IL EGF	2 732±0 233*↑	1 23±0 041**↑	73↑	33↑

TABLE 4.1 b:

Treatment	Number of days		
	WH	BL (2 mm)	DF (12 mm)
IP Control	6 (5-6)	10 (9-10)	16 (16-17)
IP EGF	6 (6-7)	7 (6-7)	13 (13-14)
IL Control	6 (6-7)	10 (10-11)	17 (16-18)
IL EGF	6 (6-7)	7 (7-8)	14 (13-15)

Treatment	Rate of growth		% increase/decrease compared to control $	
	From 2-12 mm	From 12-24 mm	From 2-12 mm	From 12-24 mm
IP Control	1 521±0 051	0.9252±0.020	-	-
IP EGF	2.064±0.169*↑	1 076±0.033*↑	36↑	16↑
IL Control	1.629±0.047	0 932±0 020	-	-
IL EGF	2 132±0 145*↑	1.156±0 024**↑	31↑	24↑

@ Values are expressed as Mean ± SE, * p≤0 05, ** p≤0 01

$ Values are corrected to the nearest whole number

Values are expressed as mode and range in parenthesis

TABLE 4.1 Number of days taken by the animals to attain various stages of tail regeneration and the rate of growth in the treated and control lizards in different stage specific treatments *viz.* before amputation (Table 4 1 a), at WE stage (Table 4 1 b) and at BL stage (Table 4.1 c)

TABLE 4.1 c:

Treatment	Number of days		
	WH	BL (2 mm)	DF (12 mm)
IP Control	6 (6-7)	10 (9-10)	17 (16-17)
IP EGF	6 (6-7)	10 (10-11)	15 (15-16)
IL Control	6 (6-7)	9 (8-9)	16 (16-18)
IL EGF	6 (5-6)	9 (8-9)	15 (14-15)

Treatment	Rate of growth		% increase/decrease compared to control $	
	From 2-12 mm	From 12-24 mm	From 2-12 mm	From 12-24 mm
IP Control	1 475±0 041	0.912±0 024	-	-
IP EGF	1 818±0 105	0 941±0 024	23↑	3↑
IL Control	1 542±0 045	0.938±0 014	-	-
IL EGF	1.796±0 075**↑	0 985±0.014	17↑	5↑

@ Values are expressed as Mean ± SE, * p≤0.05, ** p≤0.01

$ Values are corrected to the nearest whole number

Values are expressed as mode and range in parenthesis

TABLE 4.2. The nucleic acids and protein levels in the regenerates of the treated and control lizards at BL stage

Treatment	Nucleic acids and protein levels in Regenerate		
	DNA (µg/100mg protein)	RNA (µg/100mg protein)	PROTEIN (mg/100mg tissue)
IP Control	0 495 ± 0.007@	0 899 ± 0 006	0 556 ± 0 006
IP EGF	0 707 ± 0 017**↑	1.650 ± 0 004**↑	1 465 ± 0.009**↑
IL Control	0.515 ± 0 006	0 884 ± 0 010	0 548 ± 0 015
IL EGF	0 702 ± 0 015**↑	1 689 ± 0 010**↑	1 481 ± 0.012*↑

Treatment	Nucleic acids and protein levels in Regenerate	
	DNA / RNA	RNA / PROTEIN
IP Control	0 551±0 008	1.617±0 010
IP EGF	0 428±0 017**↓	1 126±0.014**↓
IL Control	0 583±0.008	1.613±0 010
IL EGF	0 415±0 019**↓	1 140±0 021**↓

@ Values are expressed as Mean ± SE, * p≤0 05, ** p≤0.01

TABLE 4 3. Variation in the nucleic acids and protein profiles between the treated and control lizards at DF stage

Treatment	Nucleic acids and protein levels in Regenerate		
	DNA (μg/100mg protein)	RNA (μg/100mg protein)	PROTEIN (mg/100mg tissue)
IP Control	0.893 ± 0.008[@]	0 417 ± 0 004	0 991 ± 0 008
IP EGF	0.984 ± 0 020*↑	0 461 ± 0 014*↑	1 057 ± 0.012
IL Control	0.896 ± 0 004	0.423 ± 0.004	0 994 ± 0 006
IL EGF	0·993 ± 0 078*↑	0 459 ± 0 007*↑	1.06 ± 0.011

Treatment	Nucleic acids and protein levels in Regenerate	
	DNA / RNA	RNA / PROTEIN
IP Control	2 141±0 021	0.421±0 005
IP EGF	2 134±0 109	0.436±0.016
IL Control	2 118±0 021	0 420±0 005
IL EGF	2.163±0.140	0 433±0 007

[@] Values are expressed as Mean ± SE, * p≤0.05, ** p≤0 01

Figure 4 1 Rate of growth of regenerate from 2-12 mm injected before amputation (Fig 4 1a), at WE stage (Fig 4 1b) and at BL stage (Fig 4 1c)

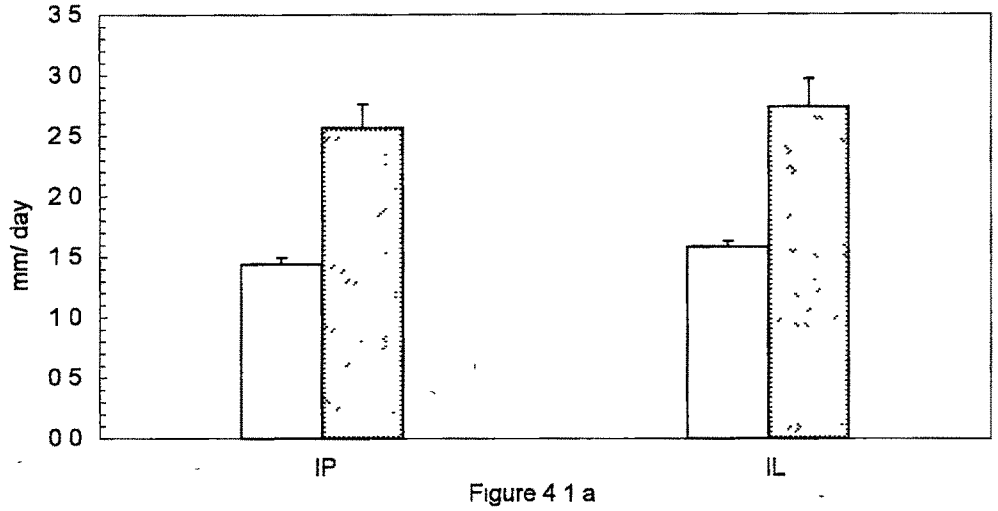

Figure 4 1 a

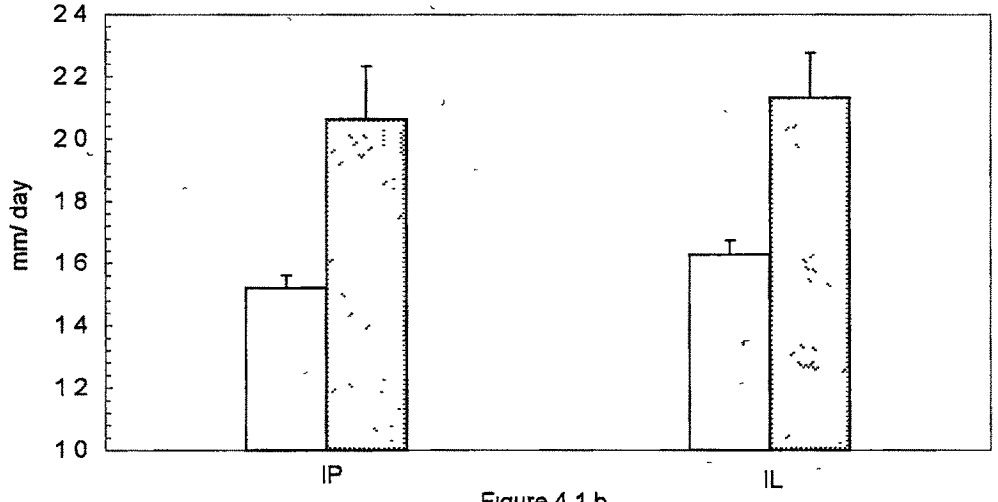

Figure 4 1 b

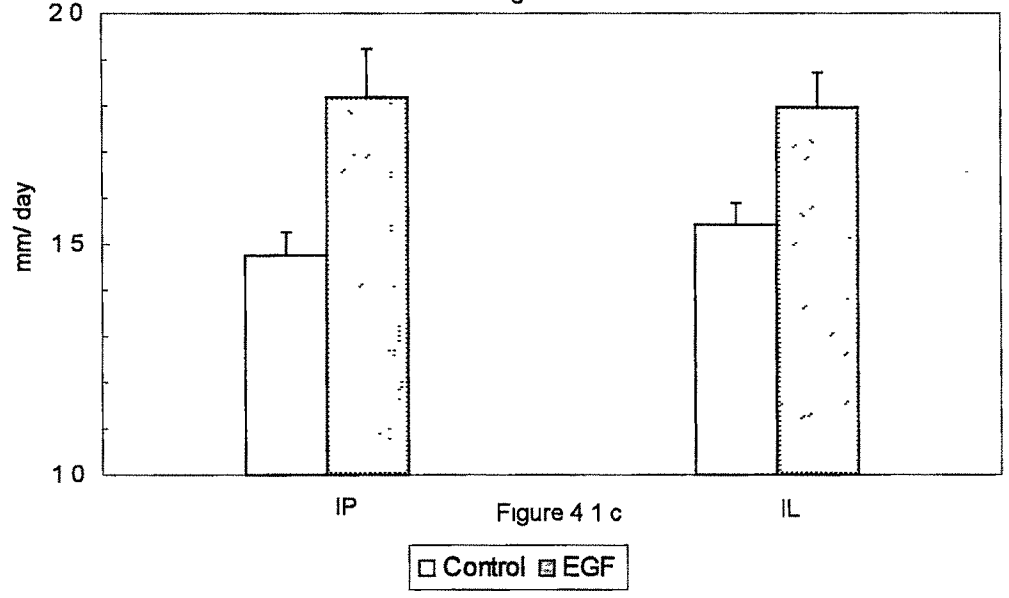

Figure 4 1 c

Figure 4 2 Rate of growth of regenerate from 12-24 mm injected before amputation (Fig 4 2a) , at WE stage (Fig 4 2b) and at BL stage (Fig 4 2c)

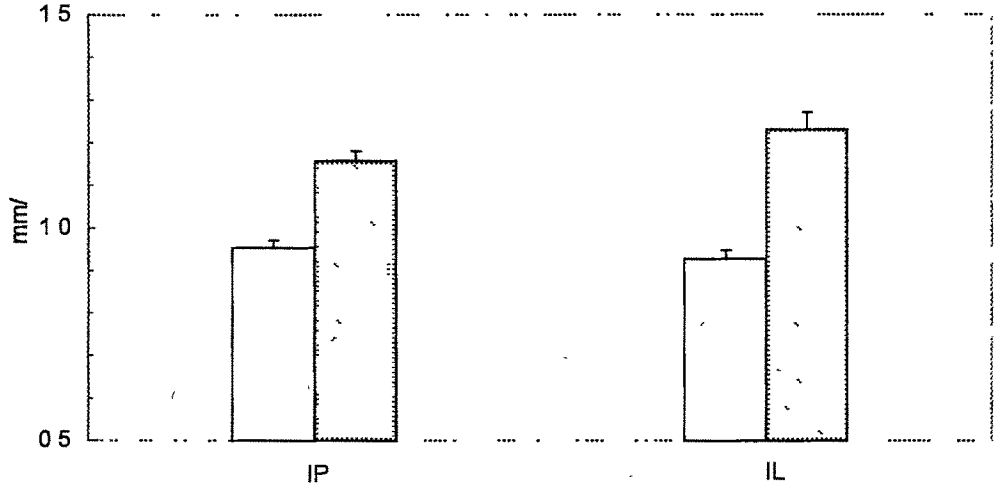

Figure 4 2 a

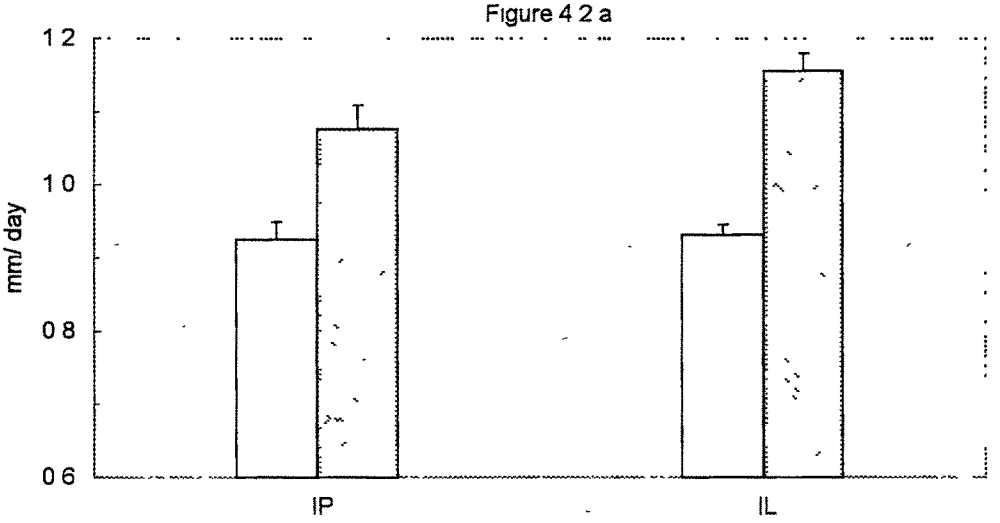

Figure 4 2 b

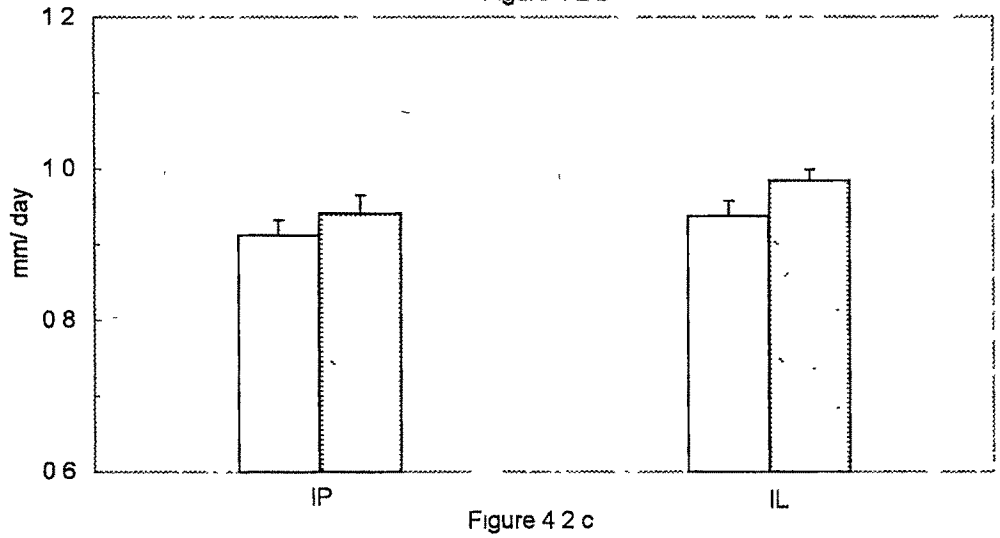

Figure 4 2 c

Figure 4.3 DNA levels in the regenerate at BL stage

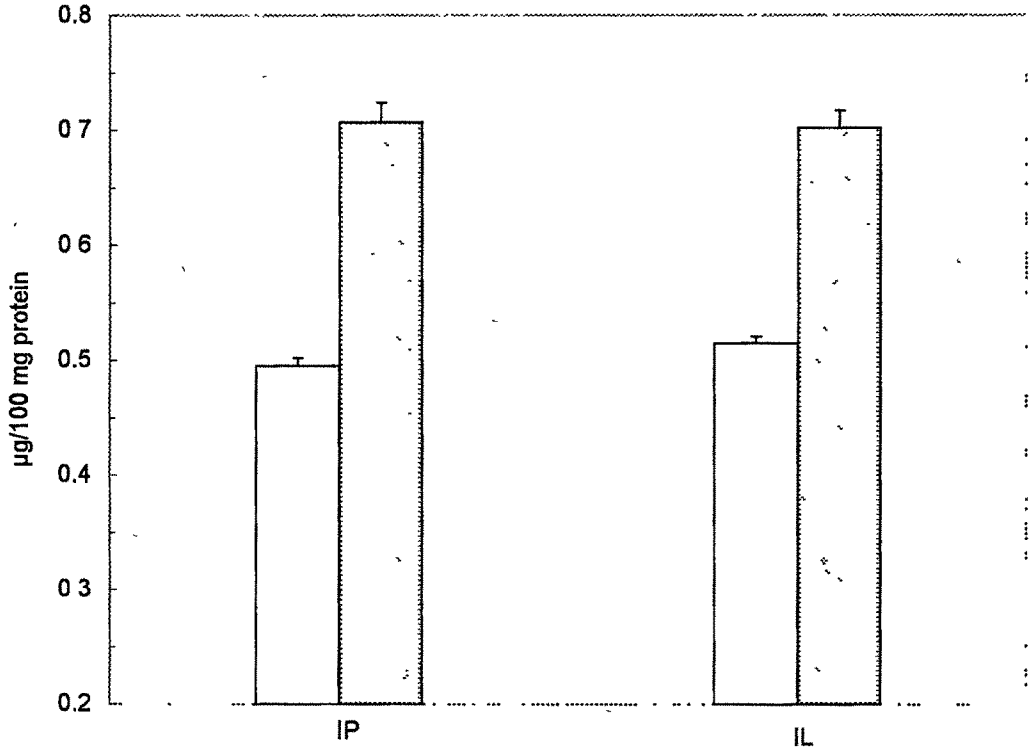

Figure 4 4 DNA levels in the regenerate at DF stage

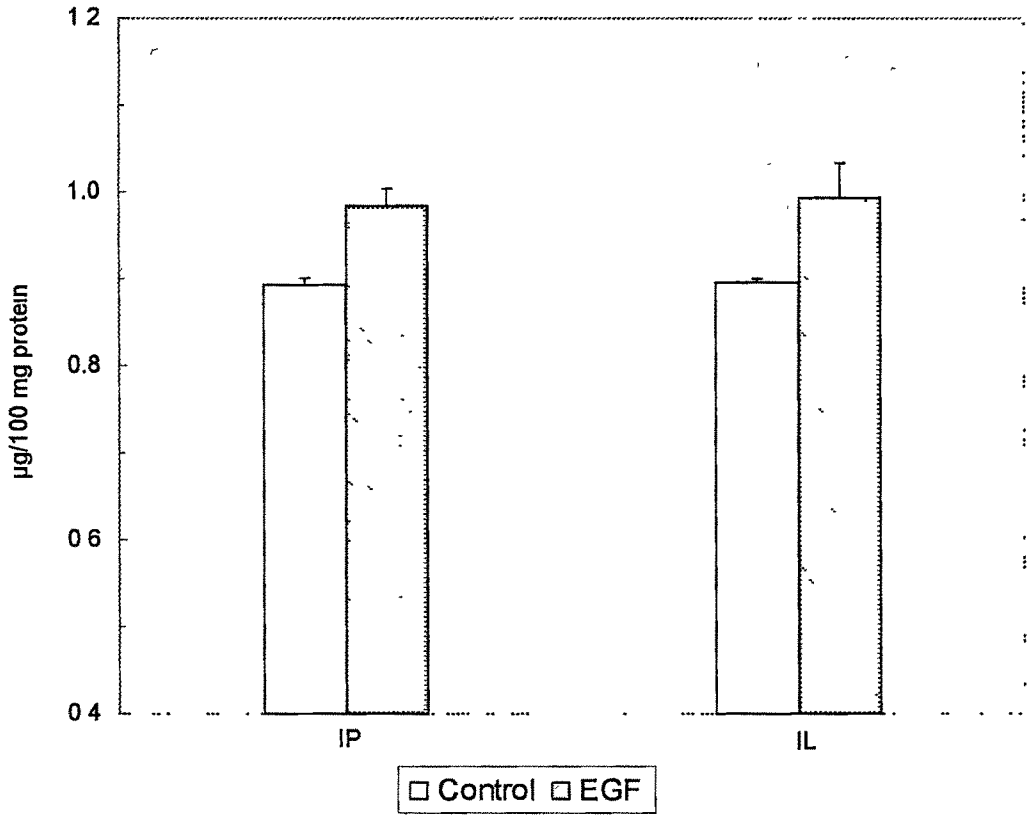

Figure 4.5. RNA levels in the regenerate at BL stage

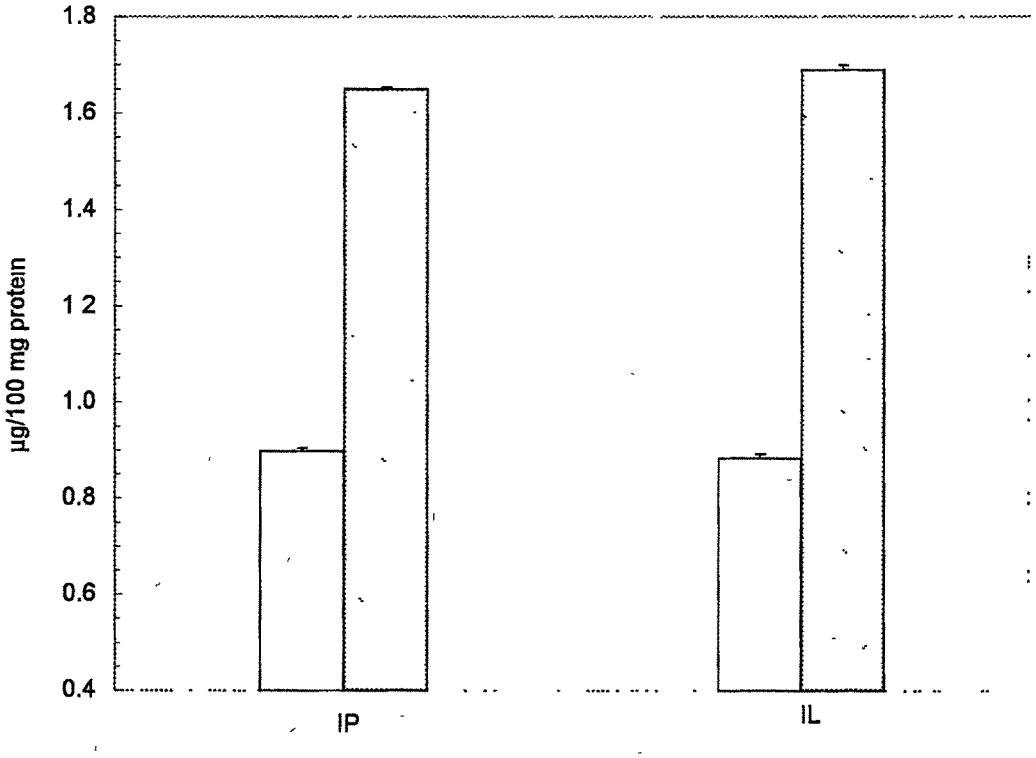

Figure 4.6 RNA levels in the regenerate at DF stage

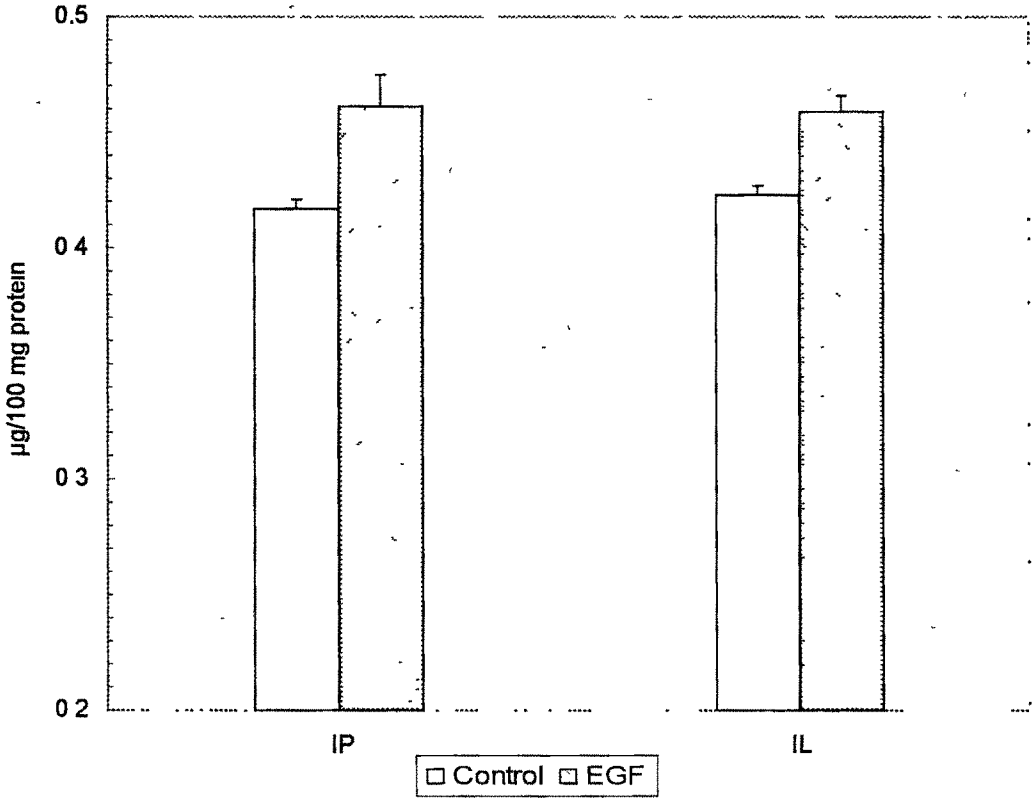

Figure 4 7 Protein levels in the regenerate at BL stage

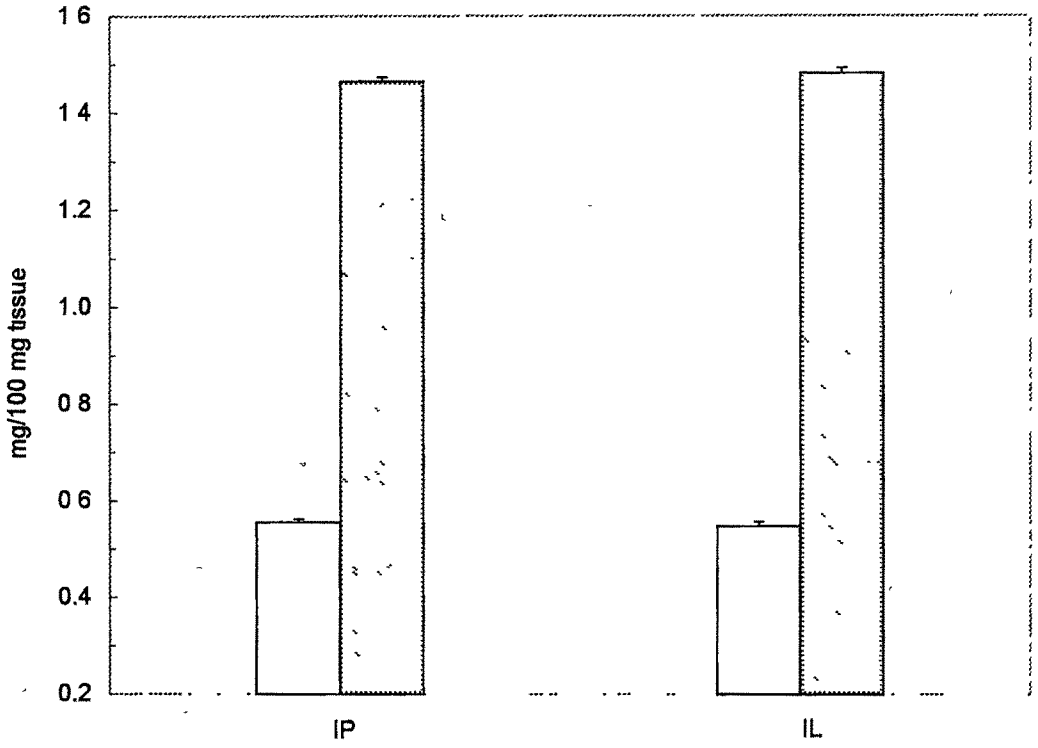

Figure 4 8 Protein levels in the regenerate at DF stage

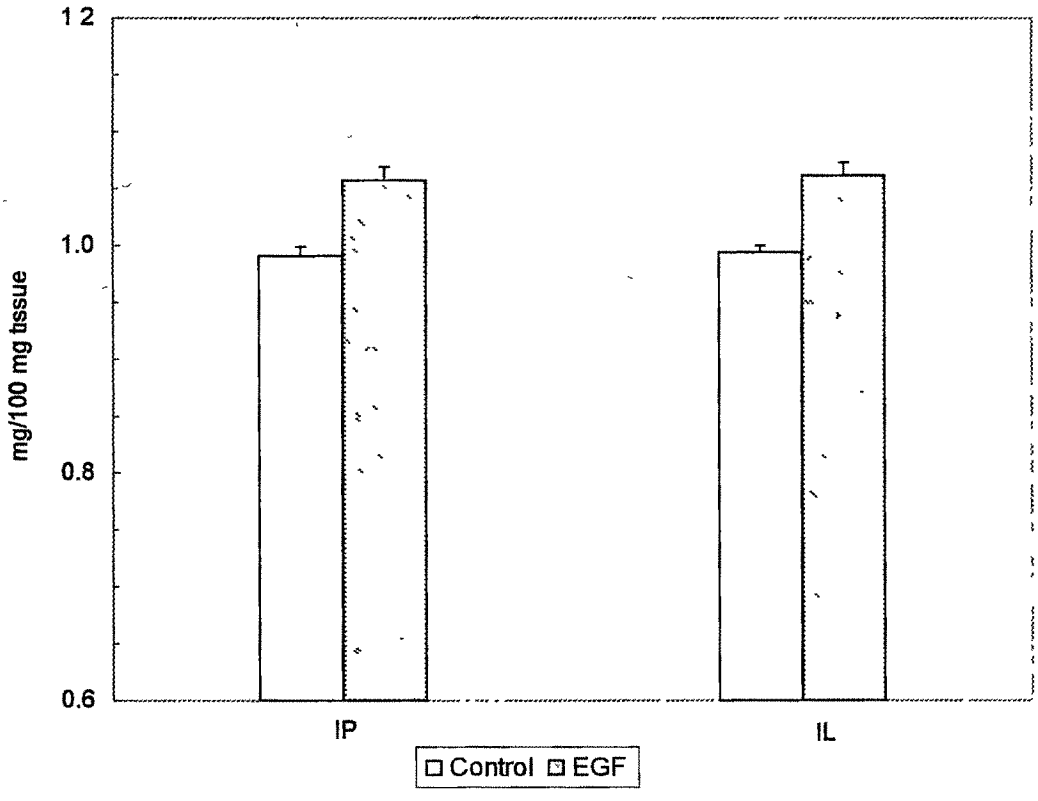

Figure 4.9 DNA/RNA ratio in the regenerate at BL stage

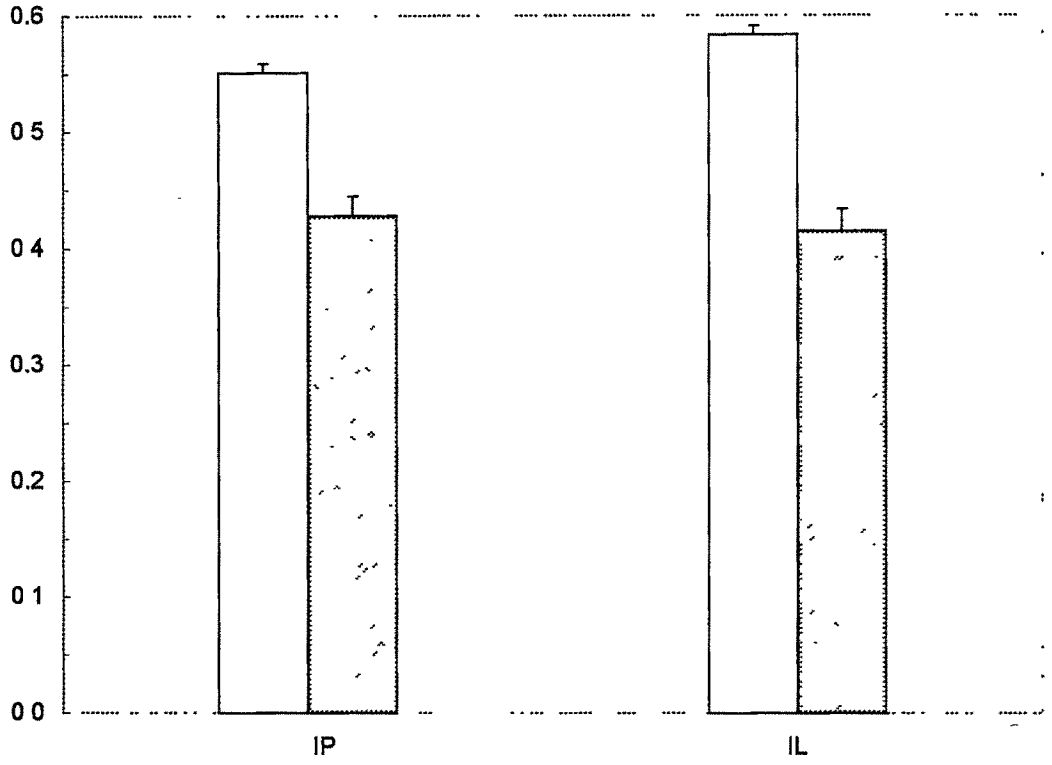

Figure 4 10. DNA/RNA ratio in the regenerate at DF stage

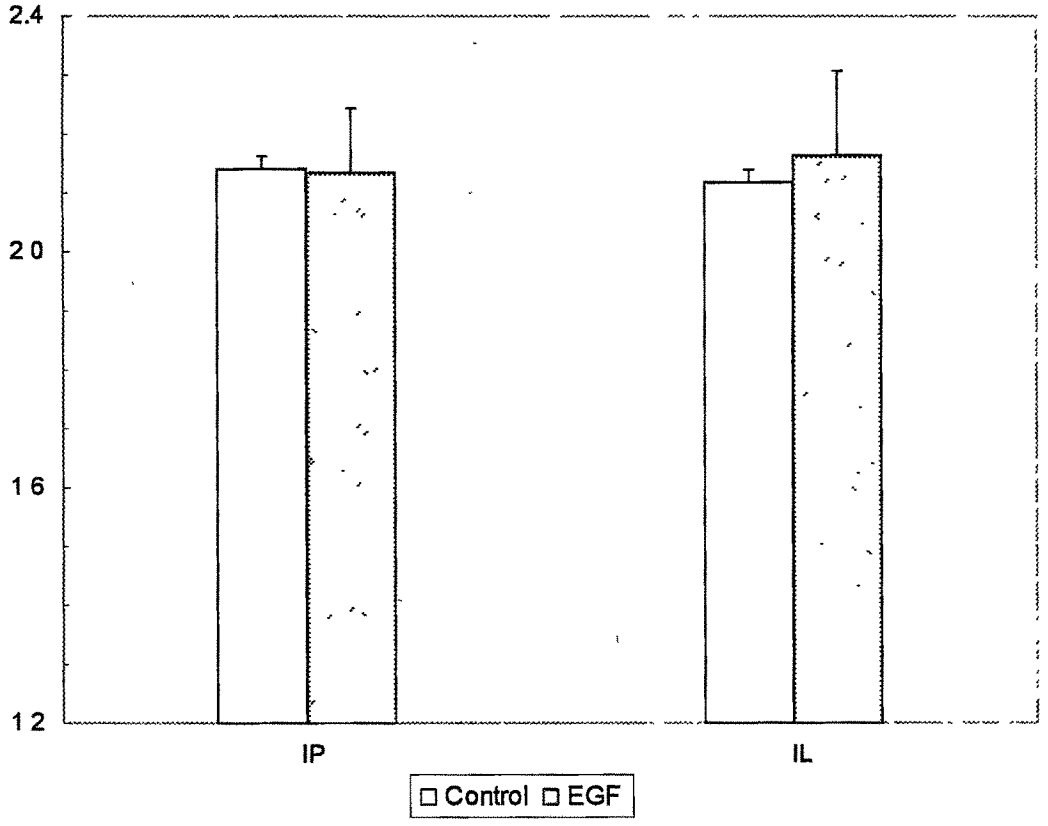

Figure 4 11 RNA/Protein ratio in the regenerate at BL stage

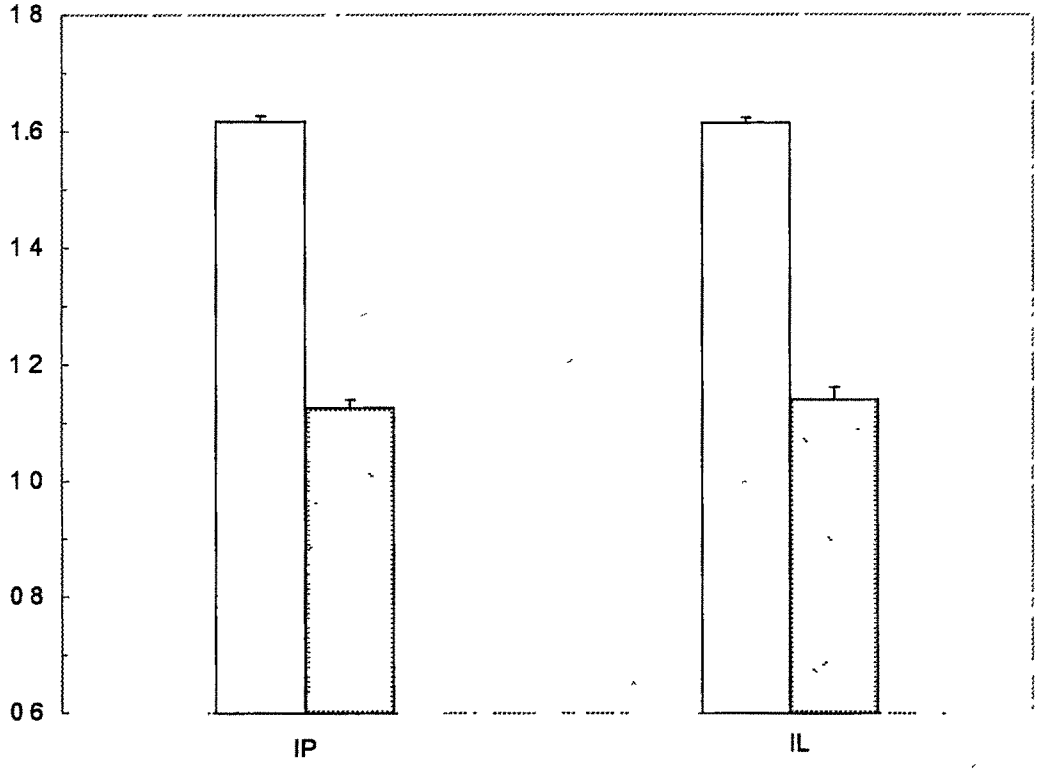

Figure 4 12. RNA/Protein ratio in the regenerate at DF stage

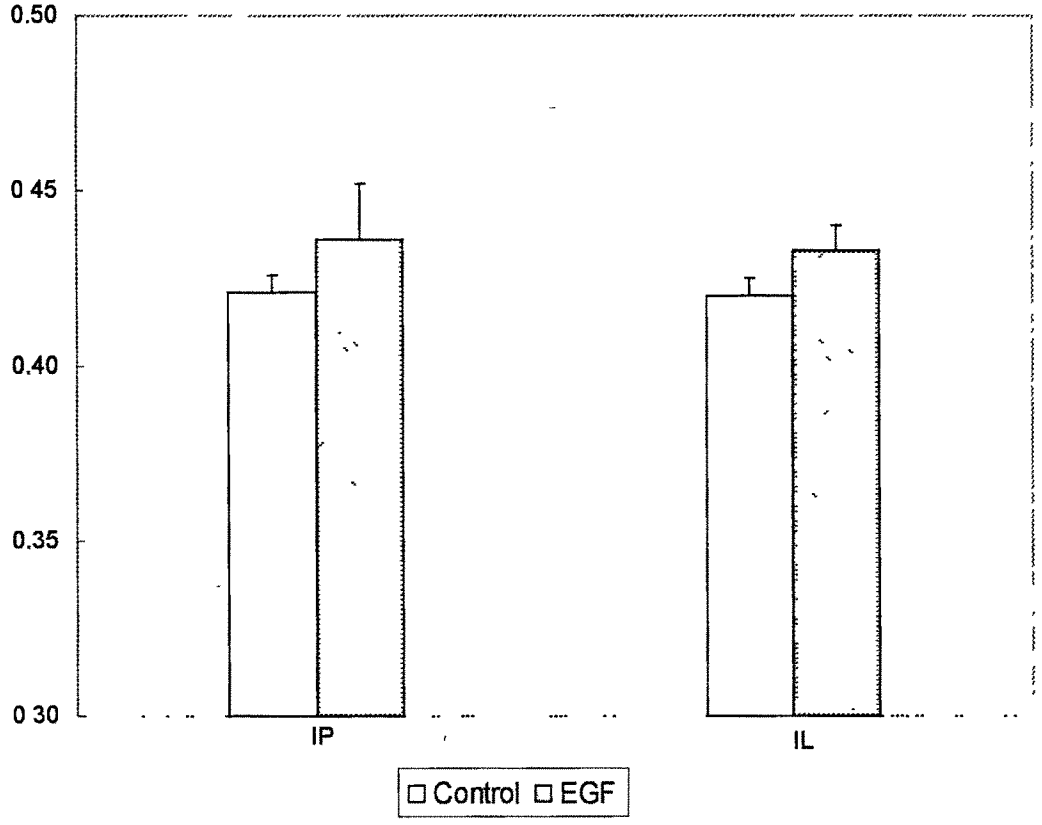

Figure. 4.13. Nucleic acid localization in regenerates of Control (Fig. 4.13a) and EGF (Fig. 4.13b) treated lizards at BL stage

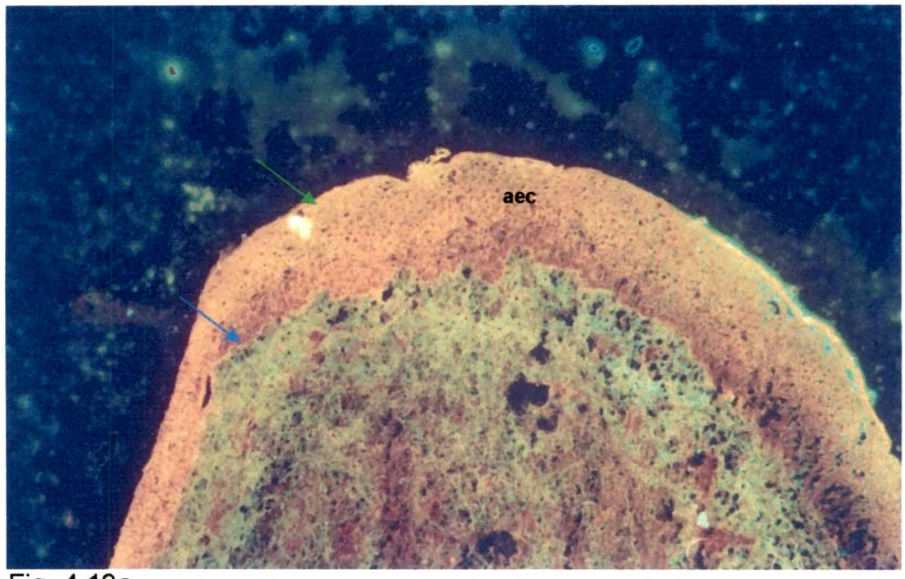

Fig. 4.13a

DNA localization → (Yellow fluorescence)

RNA localization → (Flame red fluorescence)

aec - apical epidermal cap

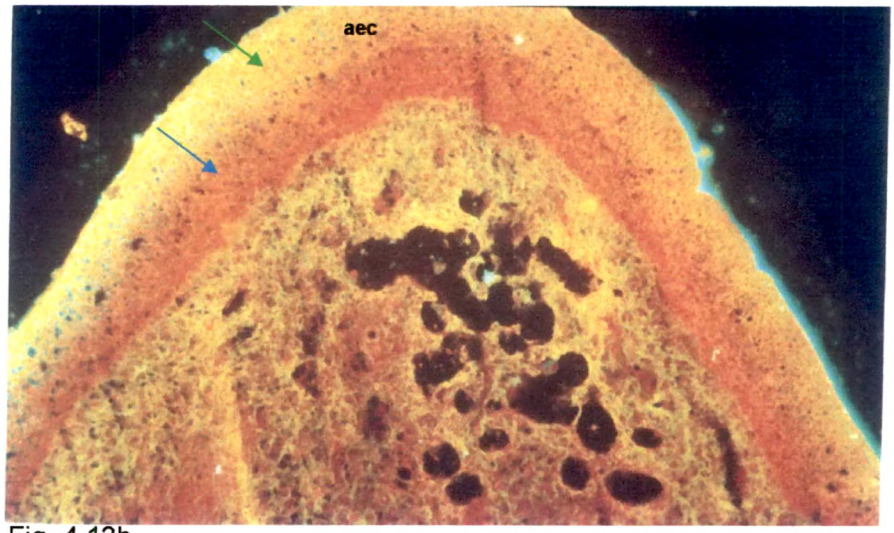

Fig. 4.13b

DNA localization → (Intense Yellow fluorescence)

RNA localization → (Intense Flame red fluorescence)

Figure 4.14. Nucleic acid localization in regenerates of the Control (Fig. 4.14a) and EGF (Fig. 4.14b) treated lizards at BL stage

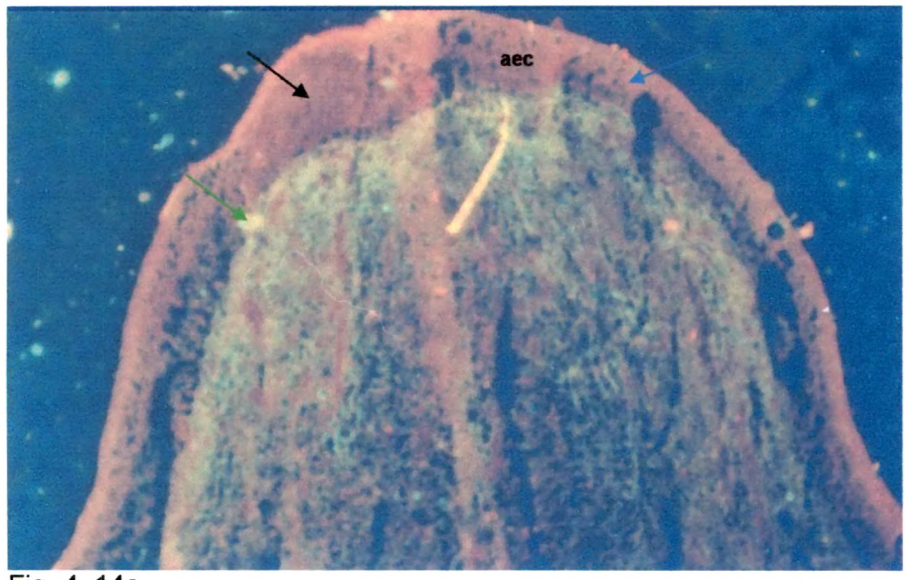

Fig. 4. 14a

DNA localization → (Yellow fluorescence)

RNA localization → (Flame red fluorescence)

Zone of active synthetic activity →

aec – apical epidermal cap

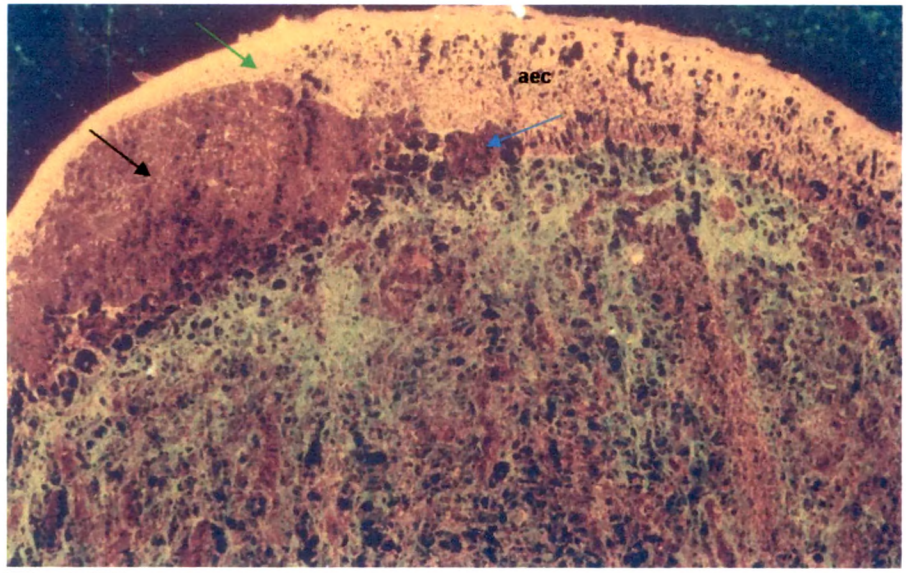

Fig. 4.14b

DNA localization → (Intense Yellow fluorescence)

RNA localization → (Intense Flame red fluorescence)

Figure. 4.15. Nucleic acid localization in regenerates of Control (Fig. 4.15a) and EGF (Fig. 4.15b) treated lizards at DF stage

Fig. 4.15a

DNA localization → (Yellow fluorescence)

RNA localization → (Flame red fluorescence)

c - cartilage tube; mm- myomeres; aec – apical epidermal cap

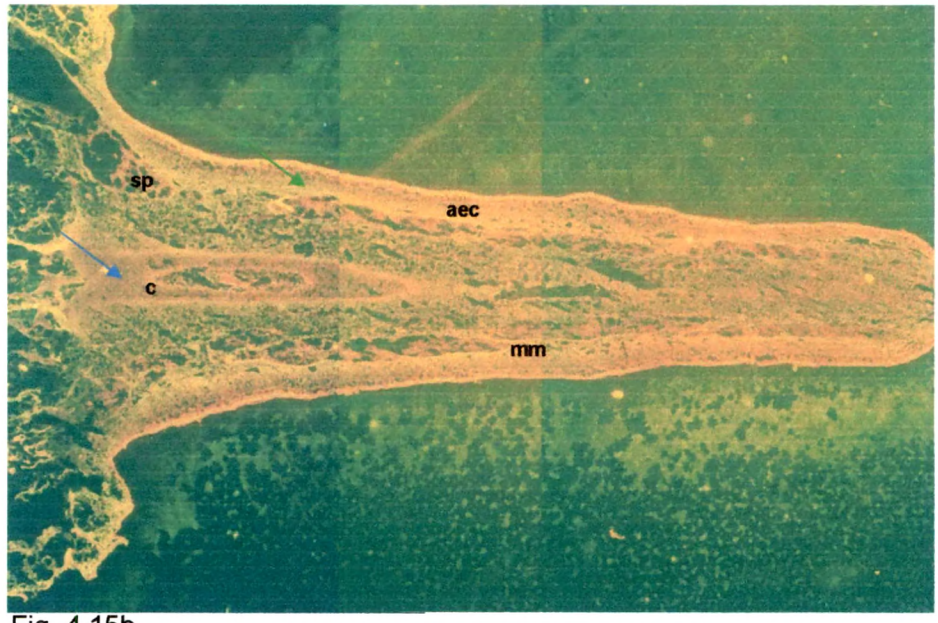

Fig. 4.15b

DNA localization → (Less Yellow fluorescence)

RNA localization → (Less Flame red fluorescence)

sp- spinal ganglia