CHAPTER - VII

CATECHOLAMINES AND GLUCOCORTICOIDS EXERT INHIBITORY EFFECT ON TAIL REGENERATION IN THE LIZARD, *HEMIDACTYLUS FLAVIVIRIDIS*: EVIDENCE FROM COMBINED CHEMICAL ADRENALECTOMY AND SYMPATHECTOMY.

The tail regeneration in lizards is accomplished through a complex series of events which are controlled by both neuronal and hormonal factors (reviewed by Shah and Hiradhar, 1978; Bellairs and Bryant, 1985). The neural contributions to tail regeneration in lizards have been investigated by Simpson(1970) and found significant contributions from the spinal cord. However, the influence of sympathetic nerves are not well attempted in tail regeneration of lizards. In the present study (chapter-II, III and IV), it has been found that catecholamines (CA) do not appreciably influence the tail regeneration in the lizard, *Hemidactylus flaviviridis*. Chemical sympathectomy in lizards found to enhance the growth rate of regenerating tail while CA supplementation retarded the process. However, a stage-specific contribution of CA during tail regeneration is suggestive from the observations that sympathectomy at preblastemic level depressed the onset of blastema formation.

Several reports are available on the metabolic alterations and variations in hormonal levels after chemical sympathectomy. In birds, it has been found that, 6-OHDA-induced chemical sympathectomy elevates the glycaemic levels and release corticosterone into circulation (Harvey *et al.*, 1984; Rintamaki, 1986; Oommen, 1992). An elevated glycaemic level was also recorded in the lizards, after 6-OHDA-induced adrenergic denervation (unpublished observations). In this context, it is hypothesized that, an excess of CORT release in turn might depress the early events of regeneration that involve a high rate of cell proliferation. Supporting evidences obtained from another set of experiments (chapter-VI), that chemical adrenalectomy enhanced the tail regeneration while CORT supplementation retarded the regenerative events. To test this assumption, the present experiments were carried out at the crucial stages; preblastemic and blastemic stages of tail regeneration in the lizard, *Hemidactylus flaviviridis*. The outline of experiment is as follows:

1..Chemical sympathectomy with 6-OHDA.
2..Chemical sympathectomy followed by reserpine administration.
3..Reserpine treatment alone.
4..Chemical adrenalectomy with Metyrapone.
5..Corticosteroid supplementation.

6..Combined chemical adrenalectomy and sympathectomy followed by reserpine treatment.

After the treatments, the growth in length of the regenerate and the level of plasma glucose were determined at specific intervals.

MATERIALS AND METHODS

As many as 250 adult *H.flavıvirıdis* were obtained locally and acclimated in the laboratory for a week on a diet of cockroaches and water was given daily. The animals were autotomised and allowed to regenerate.

Experiment-1. *Series - A:* Forty lizards which attained the WE stage on the same day were selected, divided into seven groups of 5-6 animals each and treated as follows

Group-I : The animals in this group were served as control to all the treatment groups receiving 0.6% saline only.

Group-II : A single ip. injection of 6-OHDA.Hbr at a dosage of 300 mg/kg body wt.

Group-III : Each animals received daily ip. injection of reserpine at a dosage of 5 mg/kg body wt for 4 days.

Group-IV : Animals were chemically sympathectomised with 6-OHDA as in group 1 and 6 hrs later reserpine was administered at a dosage of 5 mg/kg body wt.

Group-V : Chemical adrenalectomy was achieved in the animals by administration of Metopirone intraperitoneally in a single dose.

Group-VI : The animals in this group were given exogenous supplementation of corticosterone at 5 mg/kg body wt for 4 days.

Group-VII: The lizards in this group were chemically adrenalectomised with Metyrapone at a dosage of 200 mg/kg body wt. After 3 hrs, the animals were sympathectomised using 6-OHDA at a dosage of 300 mg/kg body wt. After 6.00 hrs, reserpine was administered at a dosage of 5 mg/kg body wt.

Series-B : Hundred and eighteen adult lizards of both sexes which attained the WE stage on the same day were divided into seven groups. The control group comprised

18 lizards, all other groups were having 15 animals. The animals were treated in the same manner as in *Series-A*. A zero hour group of animals was also maintained to determine the plasma glucose level at the WE stage. The plasma glucose levels were estimated in the control and treatment groups of 12 hrs, 24 hrs and 48 hrs and at each interval 5 animals were used. The animals were killed under deep hypothermic anaesthesia, blood was taken by cardiac puncture and plasma glucose was estimated by GOD-POD method using assay kit supplied by Stangen Immunodiagnostics, Hyderabad, India.

Experiment-2. Thirty nine lizards which attained the blastema stage on the same day were selected. They were grouped and treated as discribed in experiment-1, *Series-A*. The length of the tail regenerated was measured at 48 hrs and 96 hrs after treatments.

Statistical Analysis: The data on the length of the tail regenerated were analysed by One Way Analysis of Variance followed by Duncan's multiple range test at 0.050 significance level. Plasma glucose levels in each treatment group were compared with respect to the control group by Student's 't' test.

RESULTS

Experiment-1. (*Treatment in WE stage*), *Series-A*: The results are presented in table-1, 1.1 and fig.1. Chemical sympathectomy (6-OHDA), reserpine treatment, 6-OHDA + reserpine, corticosterone supplementation and combined chemical adrenalectomy (Metyrapone treatment + sympathectomy), all significantly decreased the growth of the tail regenerate at 48 hrs. Chemical adrenalectomy alone had no effect on the process of regeneration. Maximum growth inhibition was observed in lizards which were sympathectomised and also in those subjected to sympathectomy in combination with adrenalectomy (metyrapone + 6-OHDA + reserpine). By 96 hrs, reserpine treated or metyrapone injected animals showed no significant difference in the tail growth with that of controls.

All other treatment groups showed significant decrease in tail length with respect to the control. However, in all treatment groups, the process of differentiation was unaffected.

Series-B. The glycaemic levels recorded in different treatment groups are presented in table-2. Chemical sympathectomy with 6-OHDA produced a hyperglycaemia by 12 hrs

Table-1. Length of tail regenerated in lizards after treatment with 6-hydroxydopamine (6-OHDA), Reserpine (RES.), 6-OHDA+RES, Metyrapone (MET), Corticosterone(CORT) and MET + 6-OHDA+RES at preblastemic stage and blastemic stages of tail regeneration. The tail growth was measured at 48 hrs and 96 hrs after treatment. The tail length is presented as mean ± SD.

Treatment	Preblastemic stage		Blastemic stage	
	48 hrs	96 hrs	48 hrs	96 hrs
Control	2.56 ± 0.42	6.13 ± 0.84	4.75 ± 0.42	6.92 ± 0.80
6-OHDA	1.50 ± 0.44	3.50 ± 0.54	4.00 ± 0.89	8.33 ± 1.16
Reserpine	2.10 ± 0.22	6.80 ± 0.57	4.50 ± 0.55	8.00 ± 1.26
6-OHDA + Reserpine	2.00 ± 0.35	2.70 ± 0.27	4.00 ± 0.89	6.50 ± 0.55
Metyrapone	2.33 ± 0.52	5.25 ± 0.42	4.90 ± 0.22	8.30 ± 0.97
Corticosterone	1.90 ± 1.22	4.00 ± 0.70	4.70 ± 0.67	7.50 ± 0.50
Metyrapone + 6-OHDA+ Reserpine	1.20 ± 0.27	4.00 ± 0.35	4.80 ± 0.27	8.40 ± 0.54

Table-1.1. Analysis of growth of regenerating tail of lizards at 48 hrs (a) and 96 hrs (b) after treatment with various drugs in preblastemic stage. The data were analysed by one way analysis of Variance followed by Duncan's multiple range test. * - represents pairs of groups significantly different at the 0.050 level.

(a) 48 hrs.

	MET+6-OHDA +RES	6-OHDA	CORT	6-OHDA +RES	RES	MET	CON
MET+ 6-OHDA+ RES							
6-OHDA							
CORT	*						
6-OHDA+ RES	*	*					
RES	*	*					
MET	*	*					
CON	*	*	*	*	*		

(b) 96 hrs.

	6-OHDA+RES	6-OHDA	CORT	MET+ 6-OHDA +RES	MET	CON	RES
6-OHDA+ RES							
6-OHDA	*						
CORT	*						
MET+ 6-OHDA+ RES	*						
MET	*	*	*	*			
CON	*	*	*	*	*		
RES	*	*	*	*	*		

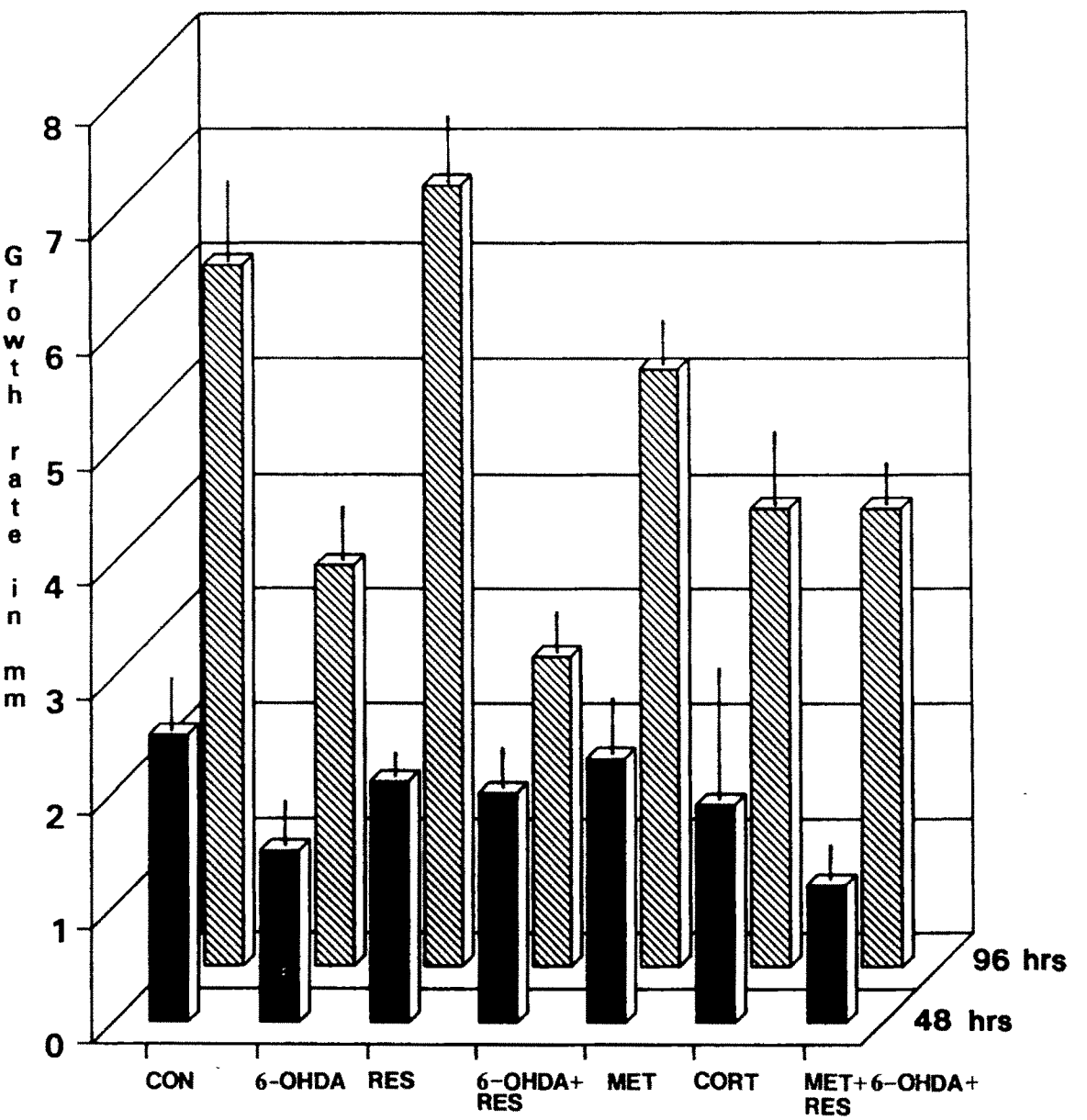

Fig.1. Length of tail regenerated in lizards after treatment with 6-OHDA, OHDA+ RES, RES, MET, CORT and MET +OHDA+ RES at WE stage. N-5-6 animals in each group.

Table-1.2. Analysis of growth rate of regenerating tail of lizards after treatment with various drugs in blastemic stage. The data were analysed by one way analysis of variance followed by Duncan's multiple range test. * denotes pairs of groups significantly different at the 0.050 level.

(a) 48 hrs.

	6-OHDA	6-OHDA +RES	RES	CORT	CORT	CON	MET +6-OHDA +RES	MET
6-OHDA								
6-OHDA+ RES								
CORT								
CON								
MET+ 6-OHDA +RES								
MET	*	*						

(b) 96 hrs.

	6-OHDA +RES	CON	CORT	RES	MET	6-OHDA	MET+6-OHDA +RES
6-OHDA+ RES							
CON							
CORT							
RES	*						
MET	*	*					
6-OHDA	*	*					
MET+ 6-OHDA+ RES	*	*					

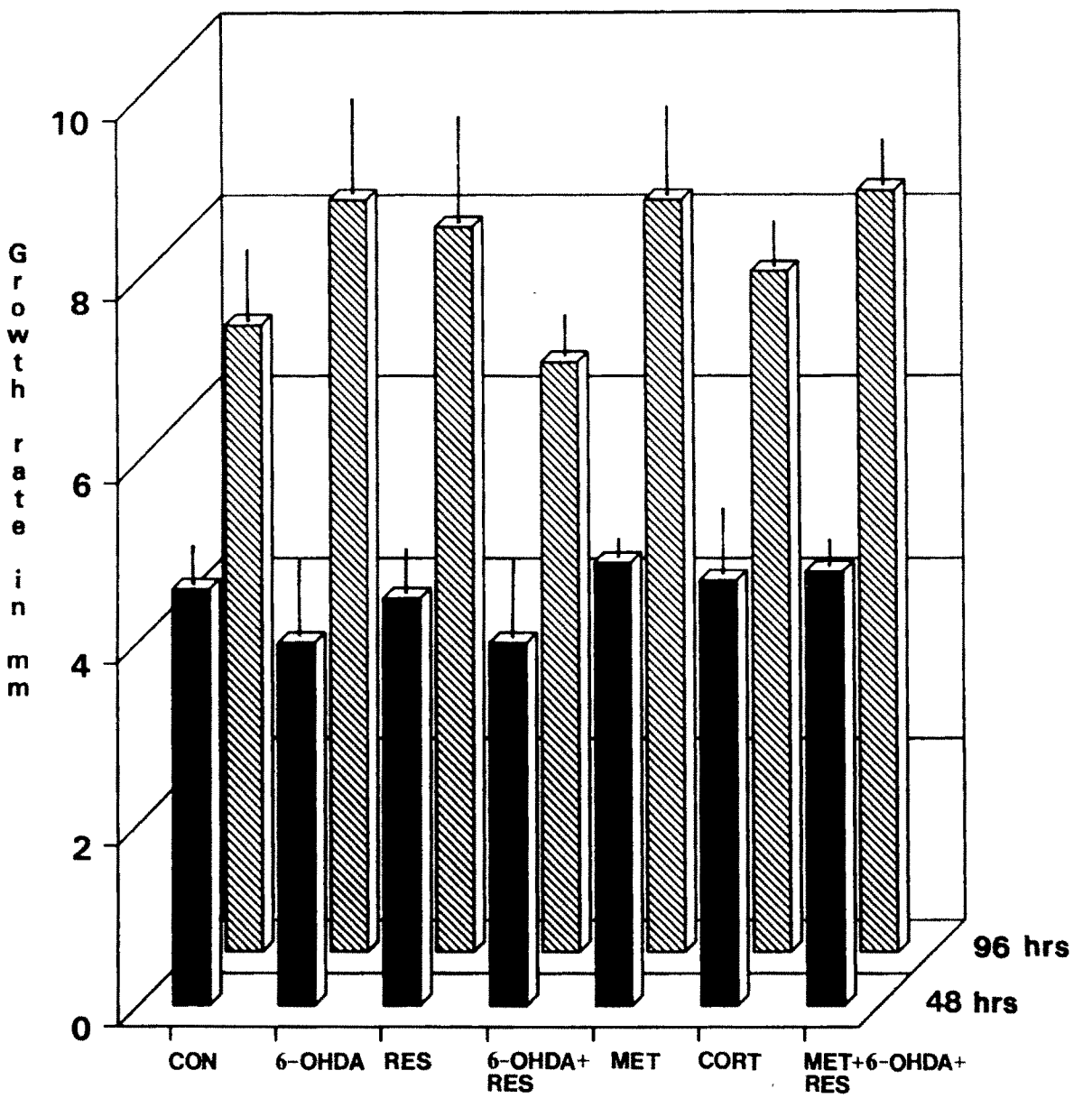

Fig.2. Length of tail regenerated in lizards treated with 6-OHDA, RES, 6-OHDA+ RES, MET, CORT and MET +6-OHDA+ RES at BL stage N-5-6 animals in each group.

Table-2. Plasma glucose levels in lizards after treatment with 6-OHDA, Reserpine, 6-OHDA+Reserpine, Metyrapone, Corticosterone and Metyrapone+6-OHDA+Reserpine at preblastemic stage (WE stage) of tail regeneration. The plasma glucose level were measured at 12 hrs, 24 hrs and 48 hrs after different drug treatments and presented as Mean ± SE. Number of animals in control group were six, all other groups five each.

Treatments	0 hrs	12 hrs	24 hrs	48 hrs
WE Stage	91.18 ± 7.65			
Control		90.30 ± 6.80	128.20 ± 4.53	125.53 ± 3.60
		**	NS	NS
6-OHDA		146.26 ± 10.2	117.22 ± 6.74	122.52 ± 9.04
		*	*	**
6-OHDA+ Reserpine		114.29 ± 4.63	108.07 ± 3.29	67.30 ± 6.03
		*	NS	NS
Reserpine		117.13 ± 7.55	130.58 ± 6.49	128.80 ± 2.54
		NS	*	*
Metyrapone		102.51 ± 3.24	103.41 ± 7.96	104.41 ± 7.74
		NS	NS	NS
Corticosterone		86.22 ± 8.63	132.72 ± 5.24	120.82 ± 6.29
		*	**	**
Metyrapone+ 6-OHDA+		112.03 ± 4.39	95.09 ± 5.62	81.49 ± 9.62

* $P < 0.05$; ** $P < 0.01$; NS-Nonsignificant

which returned to normal level by 24 hrs. Reserpine administration to sympathectomised animals decreased the plasma glucose levels resulting in hypoglycaemia by 48 hrs. Reserpine treatment alone nonsignificantly elevated the glycaemic levels. Chemical adrenalectomy produced significant decrease in plasma glucose levels which persisted till 48 hrs. Corticosterone administration slightly elevated the glycaemic levels by 24 hrs which returned to normal values by 48 hrs. Combined chemical adrenalectomy and sympathectomy with reserpine treatment elevated the glycaemic level by 12 hrs which rapidly decreased resulting in hypoglycaemia by 24 hrs. At 48 hrs also this hypoglycaemic condition persisted.

Experiment-2. (*Treatment in blastemic stage*). The length of tail regenerated in different treatment groups are presented in table-1 & fig. 2. No significant difference was observed in any treatment groups with respect to control at 48 hrs (fig. 1b) by 96 hrs, sympathectomised lizards, chemically adrenalectomised group and combined treatment group (Metyrapone+6-ODHA+reserpine), all showed significant increase in the tail growth rate with enhanced differentiation.

DISCUSSION

In control animals, a rise in glycaemic levels was observed in wound epithelium stage which attained maximum during the early differentiating stages, possibly in correlation with the increasing metabolic demands. Notable changes were observed in the glycaemic levels in different treatment groups. 6-Hydroxydopamine-induced sympathetic denervation elevated the glycaemic level resulting in a hyperglycaemia by 12 hrs that gradually decreased to normal level after 24 hrs. The present observations in lizards are similar to those recorded in birds. In birds, 6-OHDA-induced sympathectomy elevated the glycaemic level and released corticosteroids (Harvey *et al.*, 1984; Rintamaki, 1986). Administration of reserpine to sympathectomised lizards initially increased glucose levels, but by 48 hrs a low glucose level was observed. However, reserpine treatment alone increased the glycaemic level by 12 hrs which decreased to normal level by 24 hrs. Reserpine is known to release the stored CA from the nerve endings and adrenal medullary stores. Reserpine administration produced shortlasting sympathomimetic effects of NE from nerves (Maxell *et al.*, 1956; Brimijoin and Tredelburg, 1971). Thus

it is obvious that the observed hyperglycaemia is related to the increase in CA levels; the increased glucose level might have brought back to the normal level by increased secretion of insulin. Glucocorticoid inhibition produced hypoglycemia in lizards by 24 hrs while CORT supplementation sluggishly increased the plasma glucose levels. The CORT-induced elevation of glycaemic levels is well documented in reptiles (Coulson and Hernandez, 1953; Callard and Chan, 1972; Gist, 1978; Vasumati and Rangenekar, 1986). The most notable changes observed were in those lizards which were given combined treatment. Chemical adrenalectomy coupled with sympathetic denervation produced a marked hypoglycemia in lizards by 24hrs. Comparing the other treatments (6-OHDA treatment, glucocorticoid inhibition), it appears that chemical adrenalectomy and reserpine treatment together prevented the 6-OHDA-induced elevation of glycaemic levels. The above evidences clearly support the notion that glucocorticoid release occurs after 6-OHDA-induced sympathectomy which in turn might be elevating the glycaemic levels. The present results suggest that the elevation in glucocorticoid-induced hyper-glycaemia can be effectively controlled by inhibiting the adrenal steroidogenesis prior to chemical sympathectomy. Similar observations have also been recorded after the same schedule of drug treatments at blastemic stages(data not shown), which conclude that the actions of these agents are similar in all stages of regeneration.

Analysis of the tail growth rate in different treatment groups confirmed the previous observations(chapter-III & VI). Chemical sympathectomy enhanced the tail growth rate in BL stages while depressed it at WE stages. Reserpine administration could also produce inhibitory effects on growth at early regenerative events which strengthen the above findings. Chemical adrenalectomy has also been found to enhance the growth rate in lizards. These observations suggest that glucocorticoids are required in very low concentrations, probably well below the physiological levels during the process of tail regeneration. The suppression of cell proliferation observed in WE stages of chemically sympathectomised lizards may not be a direct effect of released glucocorticoids. Supporting evidences to the above concept were obtained from combined chemical adrenalectomy and sympathectomy, which could not reserve the noted inhibitory effects of sympathectomy in the early regenerative events. The combined treatment at BL stages produced a maximum enhancement in tail growth rate among the various treatment groups. This points to the fact that both CA and CORT have inhibitory effects on the differentiation stages.

On the basis of the present study, it can be concluded that both CA and CORT exert inhibitory effect upon the process of tail regeneration in lizards. However, a stage-specific sensitivity to CA neurotransmitters is likely to exist at the early events(preblastemic stages) of tail regeneration. As the cells progress through stages of division and then differentiation, sensitivity is altered probably by down or up regulation of receptors. A perfect orchestrations of neural and humoral secretions are the basis of orderliness in the progress of morphogenic process such as regeneration.