# CHAPTER - VI

# GLUCOCORTICOID INVOLVEMENT DURING TAIL REGENERATION IN THE LIZARD, *HEMIDACTYLUS FLAVIVIRIDIS*

A multiple hormone interplay is known to govern the process of epimorphic regeneration (reviewed by Liversage *et al.*, 1985). This 'hormonal milieu' is a requisite for the growth and differentiation during regeneration of newt limbs. It has been proposed that in newt limbs, the responses to a particular hormone may vary according to the specific events occurring during the periods of regeneration (Globus and Vethamany-Globus, 1985). Among various hormones that act during regeneration, the roles of glucocorticoids have been extensively investigated in newt limb regeneration (reviewed by Wallace, 1981; Liversage *et al.*, 1985). A glucocorticoid accumulation has been recorded during the limb regeneration in the newt, *Notophthalmus viridiscens* by Bromely(1977). However, ACTH replacement therapy (Tassava, 1969) or exogenous supply of corticosteroids (Schotte and Bierman, 1956) failed to restore the symptoms of hypophysectomy in regenerating newt limbs.

A few reports are available on the influence of corticosteroids during tail regeneration in lizards. In the gekkonid lizard *Hemidactylus flaviviridis*, hypophysectomy(Shah *et al.*, 1981b) and adrenal corticoid suppression using dexamethasone(Ramachandran and Abraham, 1990) have been found to retard the process of morphogenesis in regenerating tail.

The rationale behind the present experiment was to evaluate the influence of corticosteroids on tail regeneration in lizards through chemical adrenalectomy with metyrapone. Metyrapone(Metopirone, 2-methyl 1,2 D1-3-pyridyl-1-propane) inhibits the enzyme 11-ß-hydroxylase of steroid synthesis pathway and thus prevents the adrenal steroidogenesis. In another set of experiments, corticosterone (CORT) was administered in lizards during tail regeneration in order to assess the influence of exogenous CORT supplementation on the process of regeneration.

In the present study, chemical adrenalectomy and CORT supplementation were carried out in two series of experiments. In the first experiment, the hormone deprivation and supplementation were started at the pre-autotomy level and continued till 30th day post-autotomy. In the second series of experiments, the above aspects were studied at

preblastemic and blastemic stages to unravel the stage-specific functions of glucocorticoids.

## MATERIALS AND METHODS

**Experiment-1**. Twenty four adult *H.flaviviridis* of both sexes were procured from local animal dealer and acclimated in the laboratory for 7 days. The lizards were divided into 4 groups of 6 each and caged separately. The animals were fed with cockroaches and water was provided daily. The animals in each group were treated as follows.

**Group-I** : The lizards were given daily an ip.injection of Metyrapone (Sigma) prepared in distilled water, at a dosage of 50 mg/kg body wt 3 days prior to autotomy and continued till 30th day.

**Group-II** : The animals were given daily ip. injection of corticosterone (Sigma) at a dosage of 5 mg/kg body wt. The drug was prepared as suspension in 0.3% carboxyl methyl cellulose (CMC) in distilled water.

**Group-III** : The animals received ip. injection of distilled water served as control to group-I.

**Group-IV** : The animals were given an ip. injection of CMC (0.3%) served as control to group-III. In all groups, the total injected volume of drugs/vehicle was 0.05 ml per animal.

**Experiment-2**. *Series-A*: As many as 60 lizards of both sexes were autotomised and allowed to regenerate. Twenty four lizards which completed the wound healing stage on the same day were selected and divided into four groups of 5-6 animals each. They were treated as described below:

**Group-I** : Animals were injected with Metyrapone (200 mg/kg body wt) ip. in a single dosage in a total volume of 0.1 ml per animal.

**Group-II**: The animals received daily an ip. injection of CORT (5mg/kg body wt) in 0.3% CMC for 4 days (0.05ml/animal).

**Group-III**: The animals served as control to group-I, received distilled water in a volume of 0.1 ml/animal.

**Group-IV** : The animals of this group received 0.03% CMC and served as control to group-II.

*Series-B*: Twenty four lizards which attained the blastema stage on the same day were selected for the experiment. They were divided into four groups of 5-6 animals each. The lizards in each group were treated with drugs or the vehicle in the same manner as in *Series-A*. The length of the tail regenerated and the days taken to reach each stage of the regeneration were recorded. The data were analysed statistically by Student's 't' test with 95 % confidence limits.

## RESULTS

**1.Chemical adrenalectomy**: Inhibition of glucocorticoid synthesis, in experiment - 1 produced an enhancement in growth rate of the regenerative process in *Hemidactylus flaviviridis* (fig.1, table-1). The process of wound healing was completed in MET-treated lizards at an early stage. The blastema formation and differentiation of the tail was found to be advanced. Though there was an increase in the length of the tail regenerate in chemically adrenalectomised animals it was not significant when compared with that of control animals. Glucocorticoid inhibition at preblastemic stage did not affect the blastema formation though a slow rate was registered in further growth (fig.4). On the contrary, injection of MET at blastema stage significantly increased the tail length and the process of differentiation by 96 hrs. The percentage of growth enhancement in glucocorticoid-suppressed lizards were 12.5, 2, 14, 8, and 7 on days 10,15, 20,25, and 30 respectively (fig.3).

**2. Corticosterone supplementation**: Corticosterone supplementation adversely affected the process of regeneration in early stages (fig.2). The process of differentiation, wound healing and blastema formation were delayed in CORT-treated animals (table-1). The percentage of growth inhibition in CORT treated lizards were 33, 15.6, 19, 21 and 19 at days 10,15,20,25 and 30 days respectively (fig. 3).

In experiment-2 also, the above trend was recorded after CORT supplementation (table-3, fig.4). Injection of CORT at the preblastemic level retarded the process of dedifferentiation and cell proliferation. However, injection of CORT at blastema stage

Table:1. Number of days taken to reach various stages of tail regeneration in lizards treated with Metyrapone (MET.) and Corticosterone (CORT.).

| Treatment | WH | BL | ED | MD | LD | GR |
|---|---|---|---|---|---|---|
| Control | 5-6 | 7-8 | 9-11 | 12-16 | 17-25 | 25 onwards |
| MET. | 4-5 | 6-7 | 8-10 | 11-15 | 16-22 | 22 onwards |
| CORT. | 7-8 | 9-10 | 11-16 | 17-22 | 23-30 | delayed |

Table:2. Length of tail regenerated in control (CON.), Metyrapone (MET.) corticosterone (CORT.) treated lizards. The tail length is presented (in mm) as mean ± SD.

| Days | CON | MET | CON | CORT |
|---|---|---|---|---|
| | | NS | | NS |
| 10 | 4.2 ± 0.91 | 4.8 ± 0.40 | 3.6 ± 0.65 | 2.5 ± 0.54 |
| | | NS | | NS |
| 15 | 14.3 ± 1.36 | 14.6 ± 1.36 | 10.2 ± 1.92 | 8.6 ± 1.64 |
| | | NS | | ** |
| 20 | 18.0 ± 2.52 | 20.6 ± 2.13 | 15.1 ± 1.50 | 12.2 ± 1.19 |
| | | NS | | ** |
| 25 | 21.6 ± 2.73 | 23.5 ± 2.88 | 20.4 ± 1.81 | 16.0 ± 2.54 |
| | | NS | | ** |
| 30 | 25.3 ± 2.73 | 27.3 ± 3.26 | 24.0 ± 2.0 | 19.4 ± 2.76 |

** $P < 0.02$; NS - Nonsignificant.

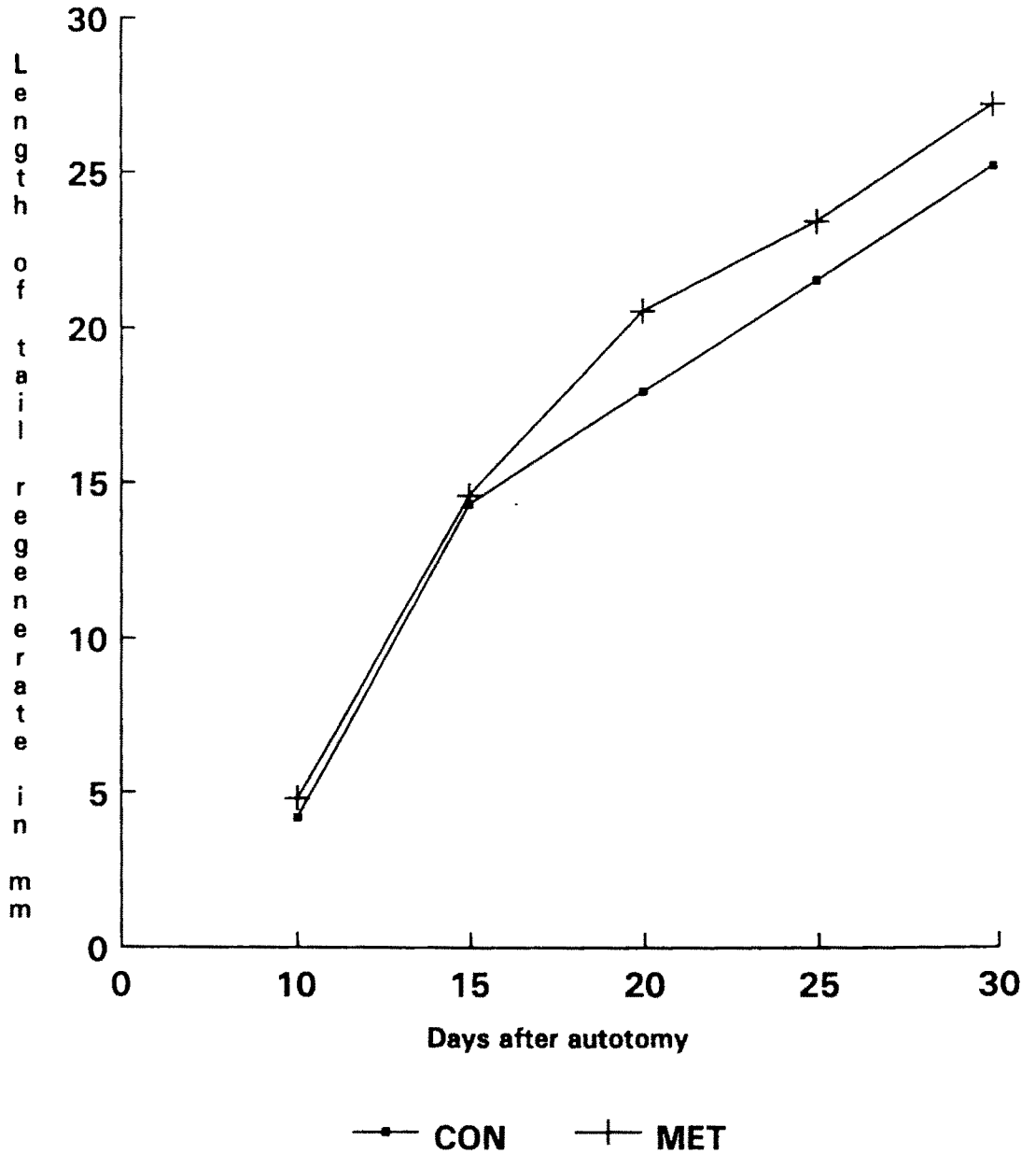


Fig.1. Length of tail regenerated in control and chemically adrenalectomised animals in a period of 30 days. Tail length is presented as mean +/- SD of 6 animals in each group.

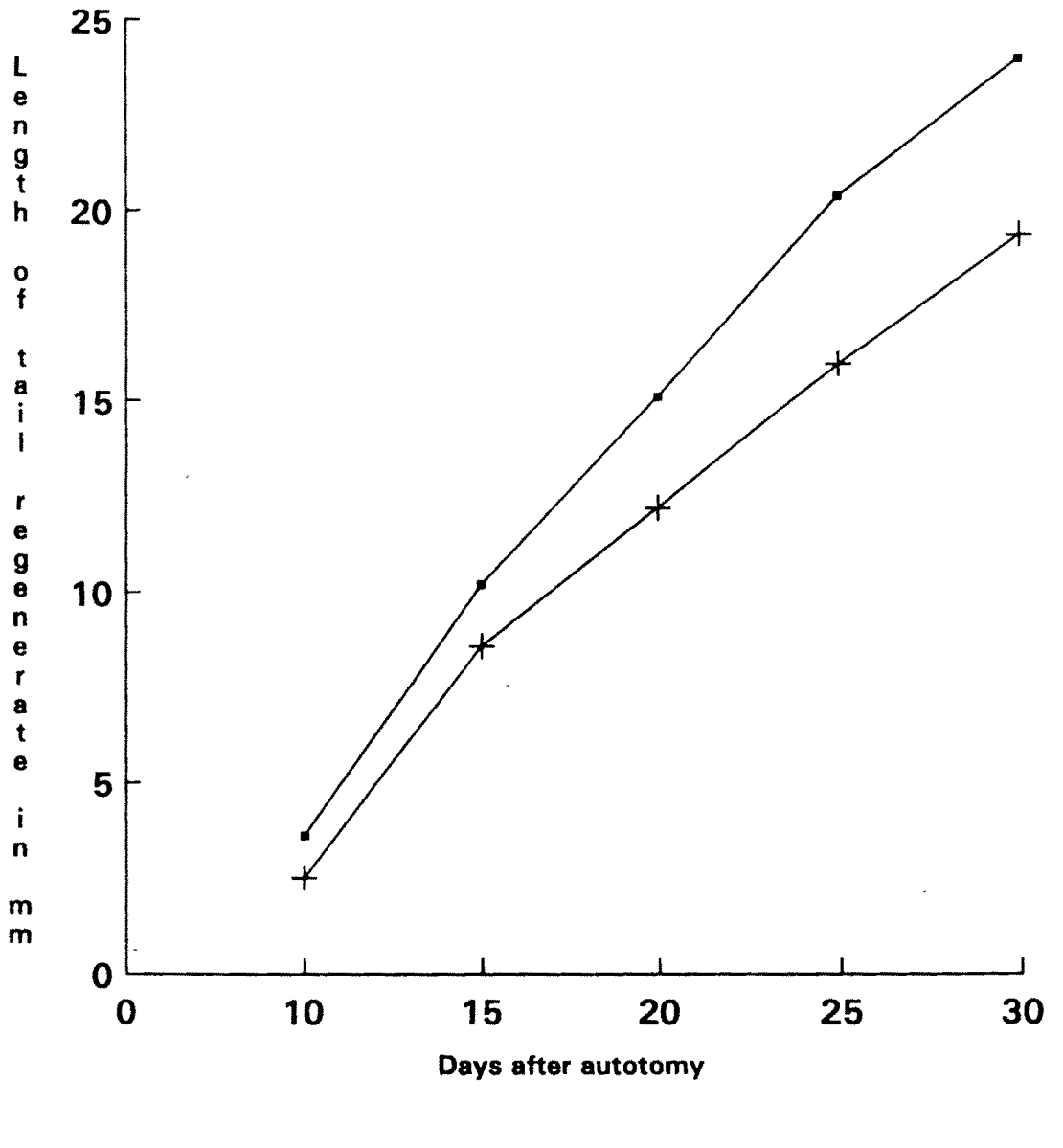


Fig.2. Length of tail replaced in lizards treated with CORT in a period of 30 days. Tail length is presented as mean +/- SD. N-6 animals per group.

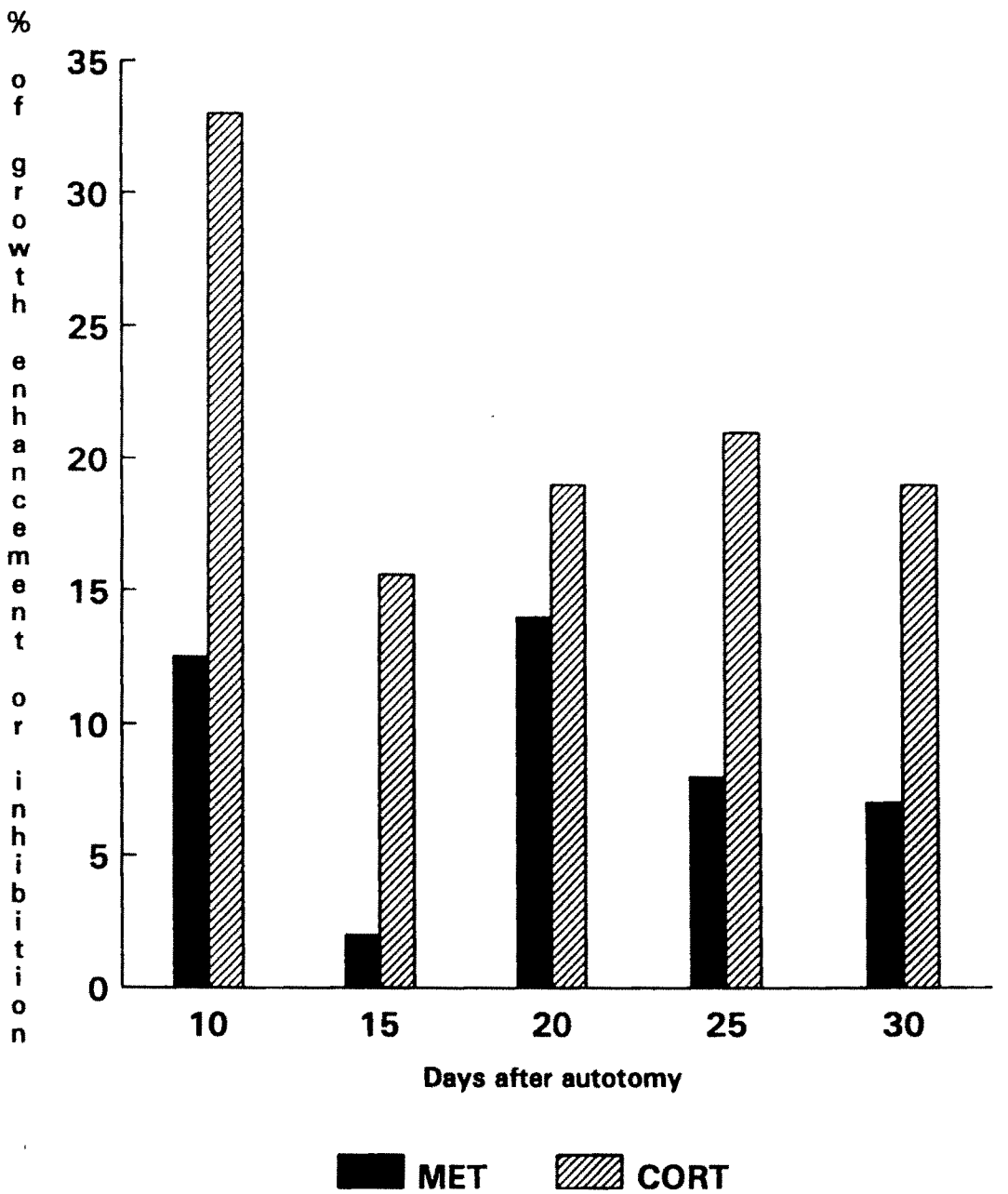


Fig.3. % of growth enhancement (MET treatment) and growth inhibition (CORT supplementation) recorded in lizards at intervals of 5 days for a period of 30 days.

Table:3. Length of tail regenerated in lizards treated with Metyrapone and Corticosterone at preblastemic and blastemic stages. The tail growth rate was measured at 48 hrs and 96 hrs after treatment. Values (in mm) are represented as mean ± SD.

| Treatment | Preblastemic stage (WE stage) | | Blastemic stage (BL stage) | |
|---|---|---|---|---|
| | 48 hrs | 96hrs | 48 hrs | 96 hrs |
| Control | 2.56 ± 0.42 | 6.13 ± 0.83 | 4.75 ± 0.42 | 6.92 ± 0.80 |
| MET. | 2.53 ± 0.52 * | 5.25 ± 0.42 * | 4.90 ± 0.22 | 8.30 ± 0.97 |
| CORT. | 1.90 ± 0.22 | 4.00 ± 0.70 | 4.70 ± 0.67 | 7.50 ± 0.50 |

* $P < 0.050$.

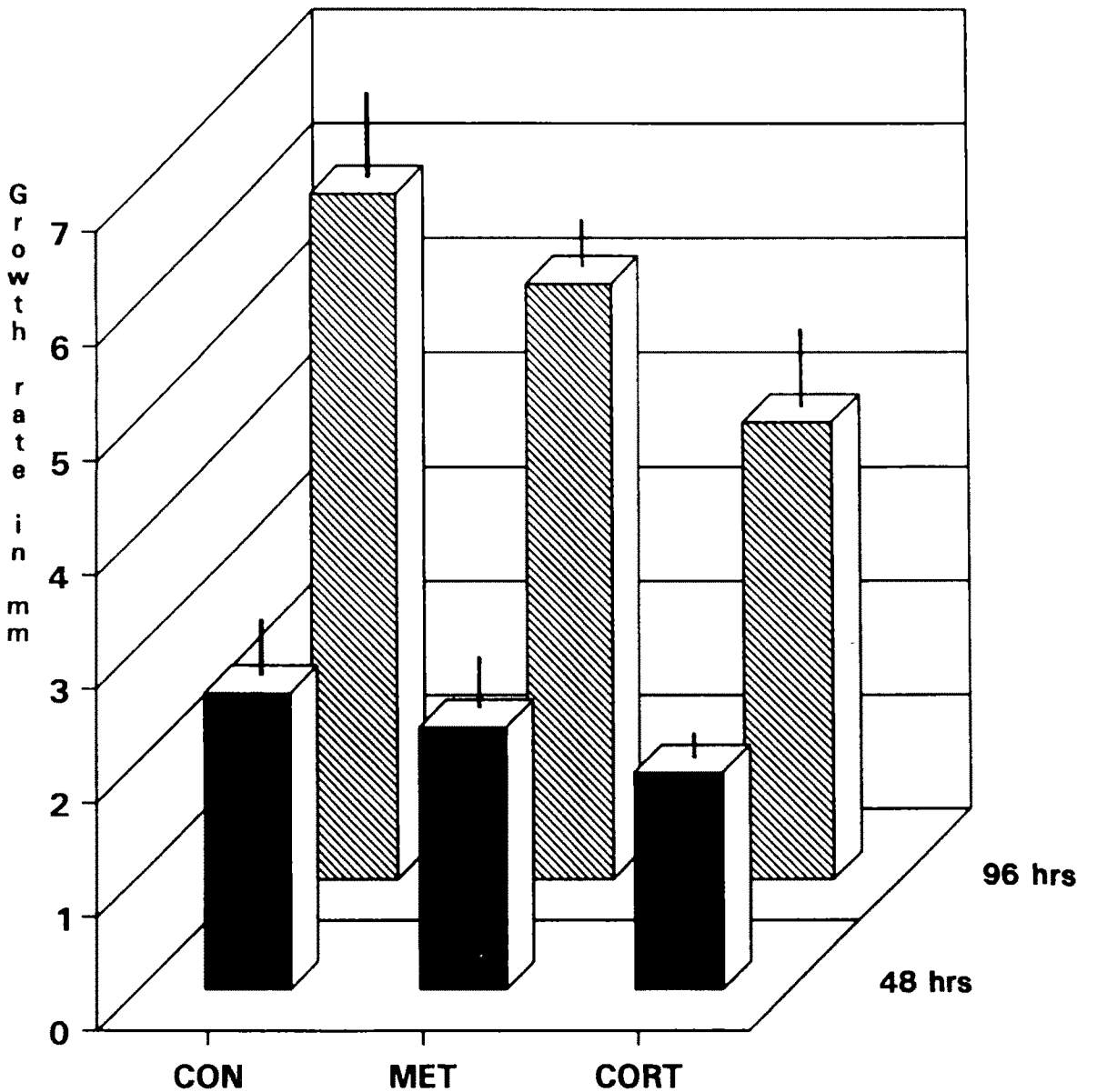


Fig.4a. Length of tail regenerated in lizards treated with MET and CORT at WE stage. Tail length was measured at 48 hrs and 96 hrs. N-5-6 animals per group.

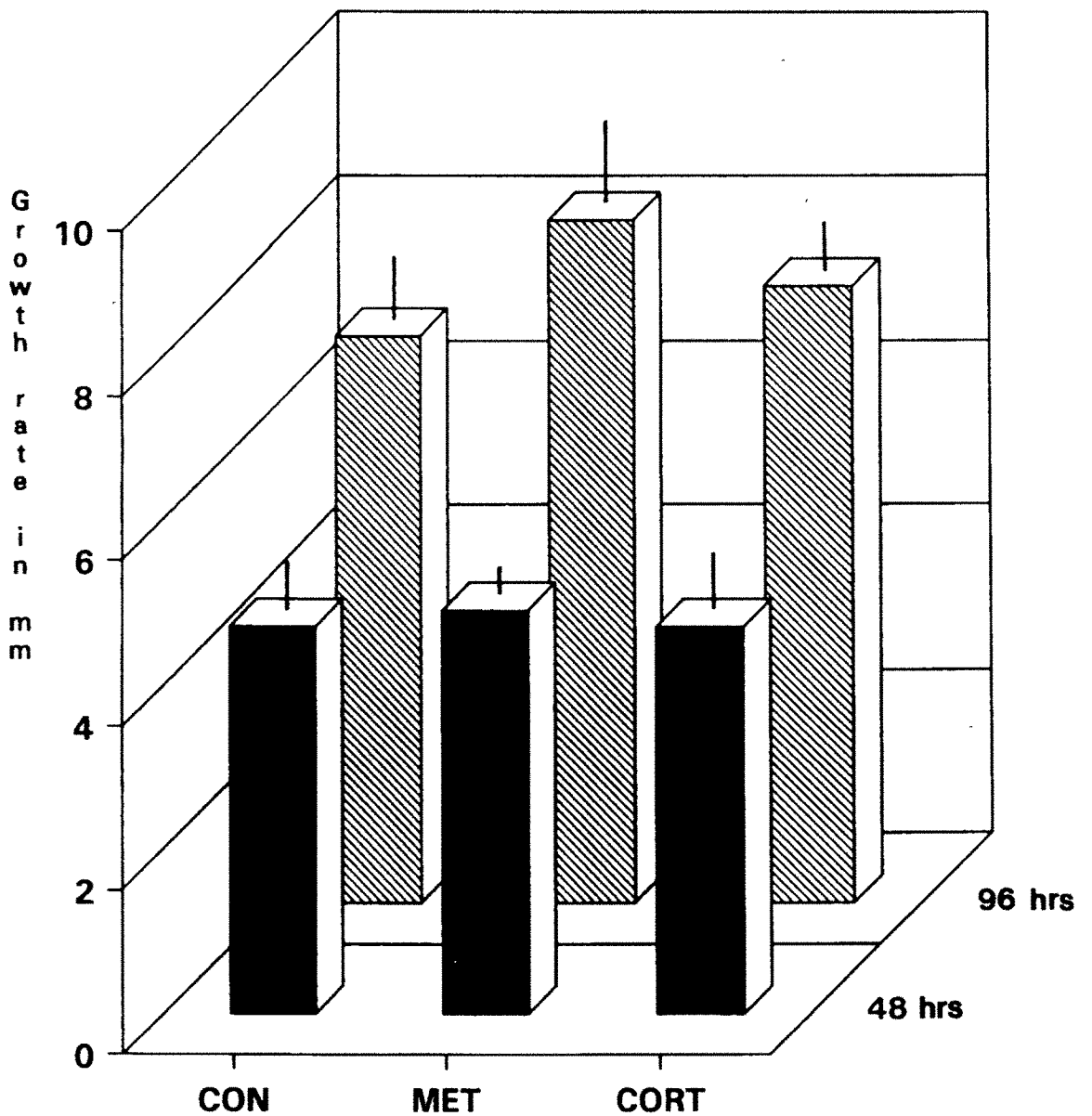


Fig.4b. Length of tail regenerated in lizards treated with MET and CORT at BL stage. Tail growth was measured at 48 hrs and 96 hrs. N-5-6 animals in each group.

did not produce any significant effect on tail regeneration in lizards.

## DISCUSSION

Glucocorticoids are known to exert both stimulatory and inhibitory effects in different organs (reviewed by Thompson and Lippman, 1974). The inhibitory effects of glucocorticoids have been demonstrated in several developing systems. In young animals administration of glucocorticoids in pharmacological dosage causes profound slowing of growth (Ingle, 1941; Winter *et al.*, 1950). DNA synthesis and growth of liver in partially hepatectomised animals are markedly inhibited by glucocorticoids (Einhorn *et al.*, 1954; Jones and Logan, 1972). The present study also demonstrates an impaired growth in tail regeneration in lizards with CORT-supplementation and a marginal increase in growth rate after glucocorticoid suppression.

A daily CORT- supplementation at 5mg/kg body wt delayed the process of wound healing while the blastema formation was unaffected during tail regeneration in lizards. As the treatment continued, the progress of differentiation greatly hampered. In contrast to this, inhibition of glucocorticoid synthesis accelerated the process of wound healing, blastema formation and differentiation of the regenerating tail. Metyrapone inhibits the enzyme 11-ß hydroxylase of the steroid synthesis pathway (Liddle *et al.*, 1958; Dominquiz and Sammuels, 1963). Metyrapone has fewer side effects than any other adrenocorticoid inhibitors (Chart and Sheppard, 1959). It has been found that in newts with intact pituitaries, the process of wound healing and epidermal cell proliferation is hampered by exogenous supply of corticosteroids; in a similar way as that seen in mammals(Manner, 1955; Williams, 1959).

Corticosteroid supplementation and glucocorticoid inhibition at crucial events, preblastemic and blastemic stages of tail regeneration, clearly exposed the inhibitory effects of CORT on tail regeneration. Corticosteroid supplementation at preblastemic level considerably slowered the dedifferentiation-proliferation events. However, the blastema formation has been found to be unaffected. Glucocorticoid suppression had no inhibitory effects on these events. The anti-cell proliferative effect of CORT are well documented in several systems. Glucocorticoids markedly inhibited the DNA synthesis in young rats by decreasing the DNA polymerase activity (Henderson and Loeb, 1970). Addition of CORT alone into cultured newt blastemata did not promote the cell

proliferation and differentiation (Vethamany-Globus and Liversage, 1973). Corticosteroids have been found to inhibit cell proliferation in cultured fibroblasts isolated from mouse embryo heart (Von Haam and Cappel, 1940). Retardation of growth was also observed in CORT treated chick embryonic limb bone rudiments (Buno and Goyena, 1955; Sobel and Freund, 1958; Fell and Thomas, 1961). Whitehouse and Lash (1961) reported reduction in chondrogenesis in chick somite treated with corticosterone. Hypophysectomy and CORT supplementation do not support the fin regeneration in the killifish, *Fundulus heteroclitus* though the survival of the animals reduced (Liversage., 1971).

Glucocorticoid inhibition at blastemic stages accelerated the process of differentiation, while CORT administration did not produce significant growth inhibition. This points to the fact that CORT might be required in a low threshold level during the process of tail regeneration in lizards. It appears that the tail regeneration can be accelerated by reducing the CORT level below the physiological level. This physiological level of CORT might be required to maintain the general metabolism and to promote the process of differentiation during the process of regeneration. Only the early events have been found to be independent of corticosteroids probably due to the fact that the regenerating cells during the early events may not be responsive to the corticosteroids. It has been found that the hormonal influence on tail regeneration in lizards are in the differentiating and growth stages (Lichet and Howe, 1969; Turner and Tipton, 1971). Also it is suggestive that during newt limb regenerating the hormones act interdependently through their effects on metabolism (Vethamany-Globus and Liversage, 1973). The differentiation during tail regeneration might be occurring under the permissive influence of glucocorticoids. Such permissive actions of glucocorticoids are well illustrated (Thompson and Lippman, 1974).

Alterations in CORT levels are conformable to cyclic nucleotide levels. Cellular low levels of glucocorticoids are shown to stimulate guanylate cyclase activity whereas increased glucocorticoid levels inhibit the enzyme activity (Vesely, 1980). Recently, it has been shown that adrenalectomy or inhibition of glucocorticoid synthesis with Metyrapone increase the NE-stimulated cyclic AMP formation in rat brain slices (Robinson and Kendell, 1990). Roberts *et al.* (1984) reported that CORT pellet implantation prevented the metyrapone-induced cyclic AMP accumulation in rat hippocampal cells. *In vitro* and *in vivo* studies in newt limbs regeneration have shown fluctuations in the ratio of cyclic AMP to cyclic GMP associated with the various stages

of limb regeneration (McLauglin *et al.*, 1983; Taban and Cathieni, 1989). However, the CORT mediated cyclic nucleotide regulation and its implications in epimorphic regeneration are not documented . It is likely that such mechanisms might exist.