CHAPTER 5

EFFECT OF HYPOPHYSECTOMY ON THE LEVELS OF ASCORBIC ACID IN LIVER, KIDNEY AND REGENERATE IN THE HOUSE LIZARD, HEMIDACTYLUS FLAVIVIRIDIS DURING TAIL REGENERATION

Ascorbic acid (AA) participates in the metabolism of living organism, whether biosynthesized by the organism or obtained exogenously through the diet. The site of biosynthesis of AA is the microsomal fractions of kidney and/or liver in various animals (Chatterjee et al., 1961; Chatterjee, 1970; Gupta et al., 1970). In general, invertebrates, fishes, some birds of higher orders, flying mammals (e.g. Pteropus medius and Vasperago abramus), guinea pig and primates appear to be unable to biosynthesize this vitamin; whereas amphibians, reptiles and birds of some lower orders have been found to possess capacity to biosynthesize this vitamin in their kidney. More evolved birds and most of the mammals biosynthesize ascorbic acid in their liver (Grollman and Lehninger, 1957; Isherwood et al., 1960; Chatterjee, 1973; Lewin, 1976).

Although most of the species have retained power to synthesize AA as a genetic trait, it is not expressed

phenotypically in all the cells of an adult organism (Fabro and Rinaldini, 1965). It has been shown in the earlier studies that the embryonic tissue has the power for the synthesis of AA during development, which gets lost during differentiation (Rinaldini, 1960; Fabro and Rinaldini, 1965). The current evidences indicate that AA biosynthesis, transport, depletion and storage are under control of hormones in vertebrates (Stubbs and Mckernan, 1967; Stubbs et al., 1967; De Nicola et al., 1968; Dieter, 1969; Biswas, 1969; Ishii et al., 1970; Bratcher and Kent, 1971; Majumder and Chatterjee, 1974). Among reptiles, both the liver and kidney of turtles can synthesize AA (Grollman and Lehninger, 1957). In lacertilia mechanism to synthesize AA lies only in their kidney (Roy and Guha, 1958).

It has been shown that AA can exercise significant influence on biological activities in various ways, viz., influencing the levels of the cyclic AMP and cyclic GMP (Lewin, 1974; Van Wyk and Kotze, 1975; Tisdale, 1975); in hormone production such as that of adrenaline, noradrenaline and serotonin (Lewin, 1976); detoxication of endogenous and exogenous toxic substances (Einhauser,1939;

Marin, 1941; Marchmont-Robinson, 1941; Mattock, 1965; Heacock and Powell, 1973); antihistamine activity (Dawson and West, 1965; Zuskin et al., 1973; Subramanian et al., 1973); phagocytosis (Cottingham and Mills, 1943; Mills, 1949; Nungester and Ames, 1948) and structural lay out of normally developing and repairing tissues (Brachet, 1950; Mazur et al., 1961; Rasmussen, 1967; Gould, 1970; Prasad, 1971; Barnes and Kodicek, 1972).

Earlier investigations on AA during reptilian tail regeneration revealed an integral association of this vitamin in the metabolic interactions and structural lay out of the regenerate (Shah et al., 1971; Ramachandran et al., 1975; Shah et al., 1976). It was thought worthwhile to determine the levels of this vitamin in liver, kidney, normal tail and tail regenerate of the gekkonid lizard, Hemidactylus flaviviridis during tail regeneration following hypophysectomy with a view to examine involvement of AA in tail regeneration.

MATERIALS AND METHODS

The house lizards, H.flaviviridis collected from the University campus were maintained in the laboratory on a

diet of young insects. The animals were kept in the laboratory for a fortnight to get them acclimatized to the laboratory conditions. Adult lizards of both the sexes in more or less same weight groups (10-12 gms) were selected. Hypophysectomy was performed through the pharyngeal approach (as described in Chapter 1). Autotomy was induced after 10 days of hypophysectomy by pinching off the normal original tail, leaving two basal segments after vent intact. Fifty lizards in each case *viz.*, hypophysectomized and sham operated ones were used for the present experiments. At specific intervals in accordance with various phases of tail regeneration, 6-8 animals per phase were sacrificed. The liver, kidney, normal tail and tail regenerates were removed and homogenized in 6% TCA (Trichloro-acetic acid) in prechilled mortars. Aliquots of the extracts were utilized for estimation of total ascorbic acid, employing the Dinitrophenyl hydrazine method of Roe (1954).

RESULTS

The levels of AA in normal tail and tail regenerates, liver and kidney of hypophysectomized and sham operated lizards, *H.flaviviridis* are presented in Tables 1,2, and 3

Table 1

Levels of ascorbic acid (AA), in the tail of hypophysectomized and sham operated lizard, H.flaviviridis, during tail regeneration.

Stages	Hypophysectomized (a)	Sham operated (b)	$\frac{a}{b}$ x 100
10 days after operation	6.783 ± 1.326 (NS)	7.492 ± 2.52	90.53
Phases of tail regeneration:			
Wound healing	35.56 ± 4.511 ($P < .025$)	43.45 ± 4.884	81.84
Blastema	33.347 ± 3.925 ($P < .025$)	27.666 ± 3.608	120.53
Differentiation	42.576 ± 6.110 ($P < .0025$)	30.456 ± 4.178	139.79
Growth (30 days)	36.012 ± 1.198 ($P < .0005$)	16.507 ± 3.357	218.16
Growth (50 days)	7.976 ± 3.218) (NS)	6.23 ± 1.545	128.02

P values in parentheses were obtained in comparison with sham operated lizards.

Values are based on 6-8 animals in each stage and expressed in mg/100g of fresh tissue as mean ± S.D.

Table 2

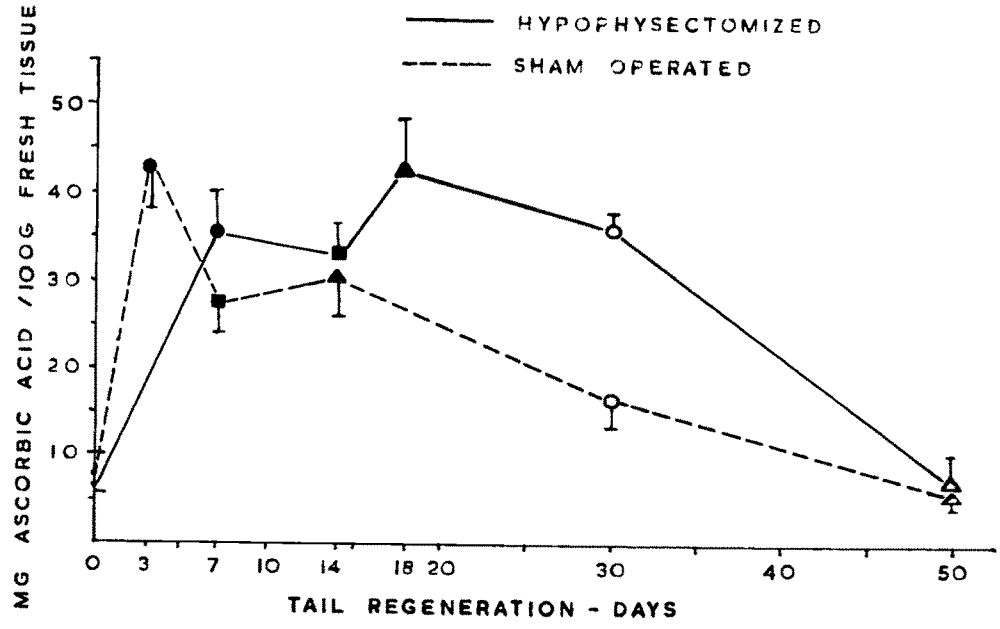
Levels of ascorbic acid (AA) in liver of hypophysectomized and sham operated lizard, H.flaviviridis, during tail regeneration.

Stages	Hypophysectomized (a)	Sham operated (b)	$\frac{a}{b} \times 100$
10 days after operation	27.55 ± 2.055 (P<.005)	21.96 ± 2.084	125.45
Phases of tail regeneration:			
Wound healing	32.816 ± 2.786 (P<.0025)	26.445 ± 0.986	124.09
Blastema	34.772 ± 3.419 (P<.01)	26.986 ± 4.489	128.85
Differentiation	52.98 ± 7.153 (P<.0025)	25.786 ± 4.380	205.46
Growth (30 days)	36.44 ± 0.957 (P<.05)	29.10 ± 7.093	125.22
Growth (50 days)	25.92 ± 6.193 (NS)	21.92 ± 4.763	118.24

P values in the parentheses were obtained in comparison with the sham operated lizards.

Values are based on 6-8 animals in each stage and expressed in mg/100g of fresh tissue as mean ± S.D.

FIG. 1


HYPOPHYSECTOMIZED
SHAM OPERATED
MG ASCORBIC ACID /100G FRESH TISSUE
10
20
30
40
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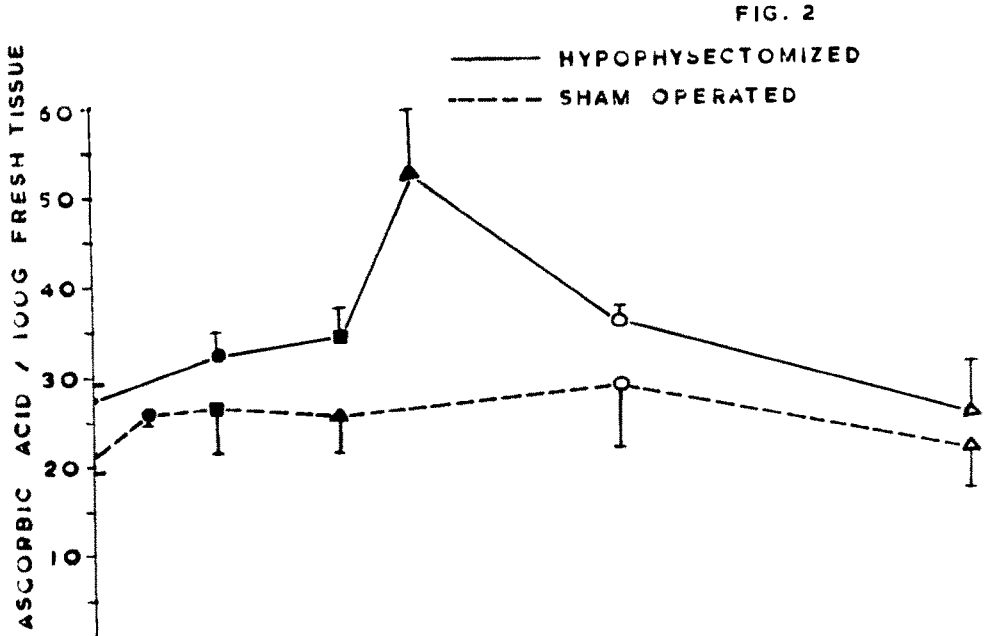
14
18
20
30
40
50
TAIL REGENERATION - DAYS

FIG. 2


HYPOPHYSECTOMIZED
SHAM OPERATED
MG ASCORBIC ACID / 100G FRESH TISSUE
10
20
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TAIL REGENERATION - DAYS

Fig. I : Graphic representation of levels of ascorbic acid (AA) in tail regenerate, during different phases of tail regeneration in hypophysectomized and sham operated lizards, H. flaviviridis.

Fig. 2 : Graphic representation of levels of ascorbic acid (AA) in liver, during different phases of tail regeneration in hypophysectomized and sham operated lizards, H. flaviviridis.

● Wound healing phase
■ Blastema phase
▲ Differentiation phase
○ Growth phase (30 days after autotomy)
△ Growth phase (50 days after autotomy)

and Figs. 1,2, & 3. There were no significant differences in the levels of AA in normal tails of hypophysectomized and that of the sham operated lizards. During wound healing, a significant rise in AA (nearly six times the preautotomy level) was observed in both the groups of experimental animals; however, the sham operated lizards showed at this stage a slightly higher level of this vitamin (43.45 ± 4.884). During the blastema phase AA concentration declined from the wound healing level in both the hypophysectomized and sham operated lizards. In hypophysectomized lizards AA concentration during blastema, differentiation and growth phases (30 days) was relatively at higher level than that observed during corresponding phases in the sham operated controls. Nevertheless, general pattern of fluctuations in AA concentration in the regenerates in both the groups of experimental lizards was similar with a difference in relative concentrations of the vitamin. With commencement of the growth, AA level in regenerate of both the groups began to decline and reached the preautotomy level when regenerates became 50 days old. However, at 30th day, AA concentration of the regenerate in hypophysectomized animals reached about twice the level (36.012 ± 1.198) than that observed in the control animals (16.507±3.357).

Table 3

Levels of ascorbic acid (AA) in the kidney of hypophysectomized and sham operated lizard, H.flaviviridis, during tail regeneration.

Stages	Hypophysectomized (a)	Sham operated (b)	$\frac{a}{b}$ x 100
10 days after operation	12.785 ± 1.198 ($P < .05$)	10.512 ± 1.894	121.62
Phases of tail regeneration:			
Wound healing	24.805 ± 4.710 ($P < .025$)	17.445 ± 3.641	142.18
Blastema	31.658 ± 4.024 ($P < .005$)	20.365 ± 2.920	155.45
Differentiation	26.805 ± 7.490 (NS)	30.273 ± 2.273	88.54
Growth (30 days)	22.156 ± 6.535 (NS)	20.496 ± 4.217	108.09
Growth (50 days)	13.355 ± 0.422 ($P < .025$)	16.59 ± 2.390	80.50

P values in the parentheses were obtained in comparison with sham operated lizards.

Values are based on 6-8 animals in each stage and expressed in mg/100g of fresh tissue as mean ± S.D.

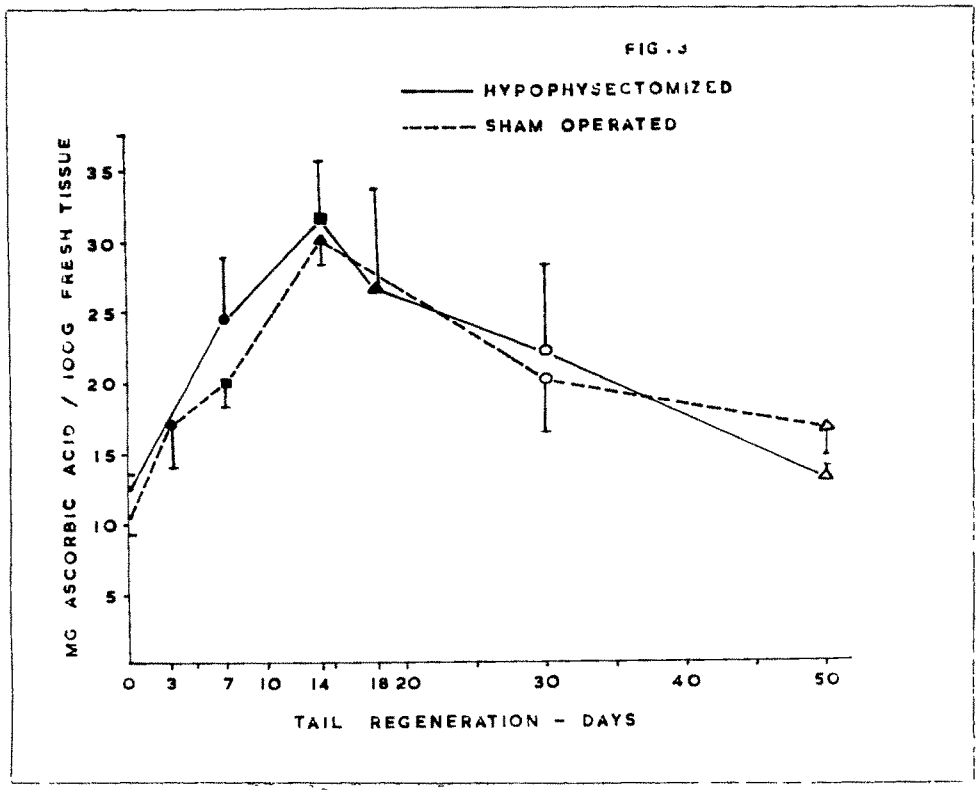

Fig. 3 : Graphic representation of levels of ascorbic acid (AA) in kidney, during different phases of tail regeneration in hypophysectomized and sham operated lizards, H. flaviviridis.

- ● Wound healing phase
- ■ Blastema phase
- ▲ Differentiation phase
- O Growth phase (30 days after autotomy)
- △ Growth phase (50 days after autotomy)

In the hypophysectomized lizards, the liver AA content began to rise with progress of wound healing and subsequent regeneration. It reached the highest peak (two fold) when regenerate was at the differentiation stage. With commencement of growth, AA contents in the liver declined to reach more or less, the preautotomy level by 50th day. As compared to sham operated lizards the hypophysectomized lizards showed higher concentration of AA in their liver during all the stages of the tail regeneration.

The kidney, where AA is known to be synthesized had a two-fold increase in the hypophysectomized and one and half times increase in the sham operated lizards as compared to their corresponding preautotomy levels during the wound healing phase. AA concentration in the kidney during blastema, differentiation and growth phases (30 days old) were significantly higher than the corresponding preautotomy levels in both the hypophysectomized and sham operated lizards. Nevertheless, by 50th day the AA level in animals of both the experimental groups returned to their corresponding preautotomy levels.

DISCUSSION

The role of AA in carbohydrate and lipid metabolism (Banerjee and Ghosh, 1947; Banerjee and Ganguli, 1962) and formation of collagen (Gould, 1963; 1970) is quite well understood. The level of AA in the original tail of the lizards 10 days after hypophysectomy has not shown any significant difference in its level as compared to that in the sham operated ones. But during the wound healing its level increased significantly (nearly six fold) in both the hypophysectomized as well as sham operated lizards. The importance of AA in wound healing during tail regeneration has been discussed by earlier workers (Shah et al., 1971; Ramachandran et al., 1975). Similarly involvement of AA in wound healing of epithelial or visceral tissues and regeneration of skin in vitro has also been studied (Ksabyan, 1956; Schauble et al., 1960; Candlish and Chandra, 1967; Prasad, 1971). Eventhough a high level of AA was seen during wound healing in the tail of the hypophysectomized lizards, the time taken for wound healing was more (7 days) as compared to that (3 days) in the sham operated lizards (Chapter 1). Such delay in wound healing observed in the hypophysectomized lizards could be due to a late attainment of the optimum concentration of AA necessary for the process.

During the blastemal phase the AA concentration declined from its previous level in the regenerate of the hypophysectomized as well as the sham operated lizards. However, the drop was quite drastic in the case of sham operated lizards. Nevertheless, the animals maintained fairly high level of AA in their blastema in both the groups of animals compared to their corresponding preautotomy values. Involvement of this vitamin during tissue repair and regeneration has been well established by earlier workers (Shah et al., 1971; Ramachandran et al., 1975). The present finding of high level of AA in the blastema of the hypophysectomized lizards could be perhaps due to some defect in its utilization by the blastemal cell in absence of hypophysial hormone/s.

The metabolic activities, during differentiation phase, when mesenchymatous blastemal cells undergo differentiation, are at peak. AA is known to be a co-factor for enzymatic hydrolization of prolyl and lysyl residues in the collagen alpha chain (Hutton et al.,1967; Schiltz et al., 1977), where proline is exclusively found in the collagen protein (Gould, 1970; Barnes and Kodicek, 1972). Increased levels of proline (Chapter 6) and the mucopolysaccharides (Shah and Hiradhar, 1975) during

differentiation have been reported wherein the implication of this vitamin in collagen formation could be suggested. Recent data have shown that high level of cAMP promotes differentiation processes (Voorhees et al., 1972; 1974; Pratt and Martin, 1975; Delescluse et al., 1976). During amphibian liver, lens and tail regeneration, a high level of cAMP was found and has been correlated with DNA synthesis (MacManus et al., 1972; Thorpe et al., 1974; Jabaily et al., 1975). It has been suggested that AA is involved in maintaining high levels of cAMP in animal tissues (Lewin, 1974; Van Wyk and Kotze, 1975; Tisdale, 1975). Since, during blastema and differentiation phases in lacertilian tail regeneration, an increase in nucleic acids (Shah and Chakko, 1972), proteins (Shah et al., 1977a) and amino acids (Chapter 6) have been observed, it could possibly be assumed that AA is involved in maintaining the required cAMP level in the regenerate, which in turn helps in the formation and maintenance of macromolecules referred above for the regenerative process. An elevated level of cAMP in the regenerating tissues of the forelimb in the hypophysectomized newt has been demonstrated (Sicard, 1975). Since AA is believed to be involved in maintaining high level of cAMP, high level of

AA observed in the hypophysectomized lizards could be considered as an advantage to this end.

As the growth of the regenerate in the hypophysectomized as well as sham operated lizards progressed, the AA level began to decline. However, its level in the 30 day old regenerate of the hypophysectomized lizards was relatively high. Though it is known that AA promotes differentiation than growth (Brachet, 1950), presently observed consistently high level of AA during differentiation and early growth phases in the tail regenerate in the hypophysectomized lizards apparently fails to prove effective, which could be so because of absence of circulating titres of the hypophysial hormones.

Mobilization of AA from distant organs of storage and synthesis during cutaneous wound healing in mammals has been investigated (Lauber and Rosenfeld, 1938; Schilling et al., 1953; Candlish and Chandra, 1967). Earlier studies on renal and hepatic concentrations of AA have shown that AA synthesis in kidney and its mobilization from liver and kidney is geared up during normal tail regeneration in house lizard, H.flaviviridis (Shah et al., 1976). High levels of AA observed in kidney and

liver of the hypophysectomized as well as sham operated lizards, during wound healing and subsequent phases of regeneration, indicates a highly geared up machinery for synthesis and storage of the vitamin in these animals as a result of the operation. Consistent maintenance of high levels of AA in liver, kidney and regenerate in the hypophysectomized lizards as compared to those in sham operated ones suggests that in absence of hypophysial hormones the involvement of AA in regeneration is considerably affected in spite of a high incidence of the vitamin in the body. It could also be suggested that such high levels of AA is probably an attempt to offset the consequent metabolic imbalance in event of lowered circulating titres of hormones with a resultant progress of wound healing and regeneration, though of poor kind.