CHAPTER 6

IN LOCO AND SYSTEMIC ALTERATIONS IN VITAMIN A CONTENT DURING TAIL REGENERATION IN THE GEKKONID LIZARD, HEMIDACTYLUS FLAVIVIRIDIS

Vitamin A palmitate promotes early post amputational regenerative changes in stumps of toad tadpoles by intensifying dedifferentiation, resulting in the formation of a good blastema (Sharma and Niazi, 1979). It has been found that in tadpoles with amputated limbs, vitamin A treatment maintains the epidermis in a larval condition, prevents dermal differentiation below the wound epithelium of the limb stumps and intensifies dedifferentiation as well as proliferation of blastemal cells derived therefrom (Saxena and Niazi, 1971; Jangir and Niazi, 1978). Increase in proteolysis is normally associated with the phase of dedifferentiation prior to regeneration and the ability of this vitamin to enhances proteolysis by its action on lysosome has been reported (Dingle, 1971). Vitamin A is also said to promote cell differentiation by mitosis (Lazarus and Hatcher, 1975). Ganguly et al. (1978) have postulated that Vit. A is required for division and differentiation of cells. They have provided evidence for this concept by using regenerating rat liver and oestrogen primed oviduct as models. DNA, RNA and protein contents were markedly less in the vitamin deficient rats and rate of incorporation of thymidine into DNA content of the oviduct was 50% of control. Hypervitaminosis of vit. A is well known to influence the morphogenesis, dedifferentiation and growth in both embryonic and adult tissues and organs, and also affect the physiological processes in a variety of ways (Aydellote, 1963; Drill, 1943; Fell, 1962; Giroud, 1956; Kalter and Warkany, 1959; Marin, 1966). These reports of involvement of vitamin A on processes integral to vertebrate appendage regeneration calls for an analysis of the quantitative content of this vitamin during regeneration. Such an analysis finds much justice in the fact that no such evaluation has been carried out during reptilian appendage regeneration. Hence, a quantitative *in loco* and systemic analysis of vitamin A has been attempted during tail regeneration in the Gekkonid lizard, *Hemidactylus flaviviridis*.

MATERIALS AND METHODS

Adult *H. flaviviridis* in the weight group 10-12 grams were collected from Baroda and maintained on a diet of insects in the laboratory. The tails were then autotomised two to three segments away from the vent. At successive intervals post-autotomy (1,3,5,7,10,15,25,40 and 60 days) the animals were sacrifised and liver, kidney and the regenerate collected weighed and vitamin A estimated according to the method described by Olson (1979).

RESULTS

Vitamin A in the liver increased during the first 3 days and then decreased on the 5th day post-autotomy. After a second increment on the 7th day, the vitamin A content of liver remained subnormal during 10th, 15th and 25th days of regeneration. Thereafter, the vitamin content increased to the preautotomy level through 40th and 60th days.

Vitamin A of the tail remained at an increased level compared to that in normal tail all throughout regeneration except on day 7th and 60th whence the levels were in the normal range.

Vitamin A in the kidney showed a slight increase on the 1st day followed by a gradual fall on the 3rd and 5th days post-autotomy. On day 7th, there was a slight increase followed by a steep and tremendous increase on the 10th day. By day 15, the level dropped to a near normal one. The level was high above normal on 25th day and sub-normal on 40th and 60th days.

Table 1. Alterations in the tissue Vit. A level (µg retinol/gm tissue) during tail regeneration in the lizard, *H. flaviviridis*.

Periods of regeneration in days	N	1	3	5	7	10	15	25	40	60
Liver	3.8421 ±0.721	7.888 ±1.54 0.001*	8.601 ±0.925	3.211 ±0.771 0.001*	8.293 ±1.562 0.0005*	2.339 ±0.487	1.805 ±0.153	1.879 ±0.057	2.834 ±0.381	3.636 ±1.201
Tail	0.1078 ±0.001	0.509 ±0.052 0.001*	1.166 ±0.087 0.01*	1.403 ±0.107	0.153 ±0.009 0.005*	0.802 ±0.421	0.686 ±0.058	1.319 ±0.077	2.209 ±0.211 0.001*	0.1166 ±0.002
Kidney	0.893 ±0.204	2.234 ±0.514	0.911 ±0.046	0.796 ±0.118	1.610 ±0.521	11.80 ±2.18 0.0005*	1.758 ±0.082	3.292 ±0.928	0.733 ±0.089	0.666 ±0.105

± S. D.

* P value

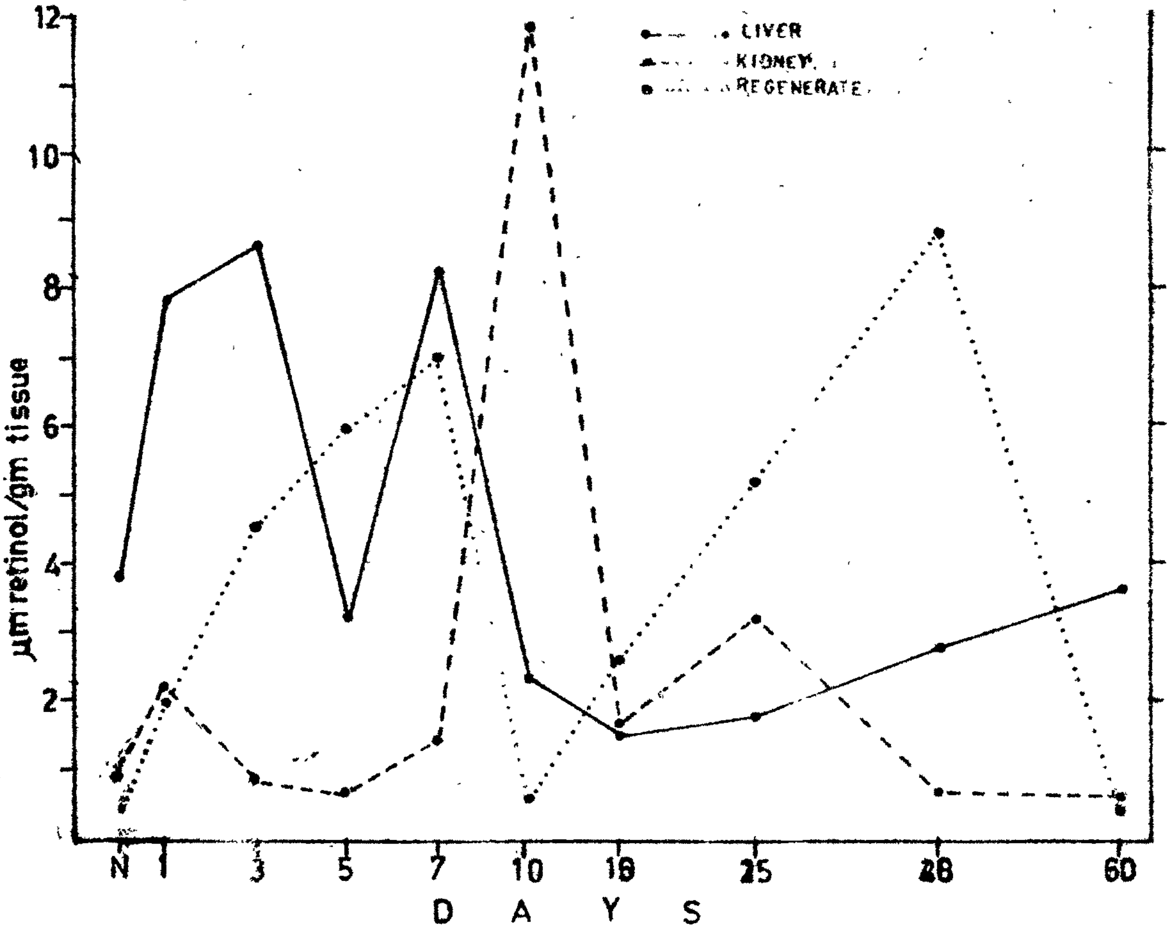

Fig. 1 : Graphic representation of the levels of Vitamin A in the liver, kidney and regenerate during tail regeneration in _H. flaviviridis_.

DISCUSSION

Liver is the principal site of vitamin A synthesis and storage, and in this light, the initial increment in hepatic vitamin A content post-caudal autotomy denotes the increased formation of the vitamin A in response to initially to autotomy and later to the regenerative stresses. Incidentally, the depletion in hepatic vitamin content correspond to the attainment of high vitamin content in the regenerate (5th day), thus indicating augmented release from liver and mobilization at the tail site respectively. The high in loco vitamin A content during the wound healing period (95%), might in the light of the reported role of vitamin A in increasing lysosomal permeability (Dingle and Lucy, 1965) and proteolytic action (Dingle, 1971) be looked upon as a favourable influence on regeneration specific wound closure and ensuing dedifferentiation. The reports that vitamin A treatment maintains the epidermis in a larval condition and prevents dermal differentiation beneath the wound epithelium (Sharma and Niazi, 1979) and intensifies dedifferentiation (Saxena and Niazi, 1977; Jangir and Niazi, 1978) in the limb stumps of anurans are very relevant in the present context. 5th to 7th day which corresponds to the blastemic phase is marked by a second spurt in synthesis of the vitamin in the liver and depletion in the regenerate. While the decreased content in the regenerate could denote its greater utilization then supply in the late dedifferentiative activities, the increment in the liver is suggestive of lower rate of its release stockpiling for meeting the requirements during blastema and differentiation phases of regeneration. Such a conclusion is substantiated by the depletion of hepatic vitamin A content between the 7th and 10th days and the maintainence of a sub-normal plateau level thereafter till about the 40th day. Concomitantly the vitamin content in the regenerate increased to an above normal level in the blastema phase and this high content was maintained thereafter all throughout differentiation. Supra normal levels of vitamin A in the regenerate during the blastemic and differentiation phases speak of an intimate association of this vitamin with the many events characteristic of these phases of regeneration. Blastemic and immediate post-blastemic phases are characterised by increased gene activity, mitosis, transport of materials and ions and differentiation; and vitamin A could be implicated in many of these aspects as the relevant literature indicate. Some of the pertinent reports to this end are those of Tsai and Chytil (1978) of involvement of vitamin A in transcription, Lazarus and Hatcher (1975) and Ganguly et al. (1978) of its role in cell proliferation and differentiation and of Bunting (1967; 1969), Conard et al. (1974) and Stillwell and Rickett (1980) of its importance in permeability and transport of ions and organic molecules. Further, vitamin A has also been implicated in the biosynthesis of glucosaminoglycans, glycolipids and glycoproteins (Dusheiko et al., 1978) while, retinyl phosphate has been hypothesized to act as an intermediate in the transfer of sugar moieties from nucleotide sugars to the growing oligosaccharide chains of glycoproteins (Masuchigi et al., 1978) and, retinol to stimulate the formation of glycophospholipids (Rosso et al., 1975). Apparently, all these activities are of crucial significance in the structural layout of fast proliferating and differentiating cells of the regenerating tail. Thus, multiple involvement of vitamin A during the blastema and differentiation phases of regeneration can easily be visualized.

What is more interesting is the tremendous increase of the vitamin content in the kidney during the blastemic and immediate post-blastemic periods of tail regeneration. As is evident from the figure 1 the peak content of the kidney corresponds to the depleted levels in the regenerate and liver. Subsequently, the complete depletion of the increased kidney vitamin content between the 10th and 40th days is marked by the concomitant attainment of a maximal level in the regenerate. Presumably, the <u>in loco</u> increased requirement during the differentiation and growth phases is catered to by the kidney, for which the organ mobilized and stored a large quantities of the vitamin during the blastemic phase.