CHAPTER 2

LOCAL AND SYSTEMIC ALTERATIONS IN PROTEIN CONTENT POST-CAUDAL AUTOTOMY AND POST-LIMB AMPUTATION : A COMPARATIVE EVALUATION IN HEMIDACTYLUS FLAVIVIRIDIS

The most important biochemical process representing growth, is the biosynthesis of proteins; and the nucleic acids (DNA and RNA) have an essential role in this process (Rappaport and Fritz, 1972). Brachet (1947, 1950a, b) considered the functions of nucleic acids in regeneration to be essentially the same as during ontogenesis. The importance of nucleic acids in amphibian regeneration has been related to increased cell metabolism, growth and protein synthesis (Yakovleva, 1943; Clement Noel, 1944; Roskin and Karlova, 1944; Litwiller, 1939; Hay and Fischman, 1961; O'steen and Walker, 1961; Riddiford, 1960). Thornton and Bromley (1973) opined that protein metabolism gets extensively geared up in accordance with regressive (dedifferentiation) as much with the progressive phases (blastema formation, differentiation and growth) of regeneration. Apart from the above reports on amphibians, studies conducted on reptiles in this laboratory (Shah and Chakko, 1969; Radhakrishnan, 1973; and Ramachandran *et al.*,

1980) have emphasized the significance of nucleic acids and protein *in loco* as well as systemically during tail regeneration in lizards. With the establishment of definite local and systemic responses in the regenerative mechanics in lizards, it was pertinent to compare the local and systemic responses that occur during tail regeneration with that occurring during post-limb amputation as the limbs in lizards do not have the regenerative ability. Such studies, it is hoped, would enable us to understand the possible causative factors for the loss of regenerative ability in other extremities of the body (limbs) other than the tail. This thinking has prompted the present comparative study on *in loco* and systemic alterations in protein content during post-autotomy caudal regeneration and post-limb amputation.

MATERIALS AND METHODS

H. flaviviridis collected from Baroda were kept in the laboratory for a fortnight to get them acclimatized to the laboratory environment. The animals were kept on a diet of insects. The lizards were divided into two groups : one, with their tail autotomised two-three segments from the vent, and the other, with their forelimb amputated from the distal part of the humerous. Lizards from both the groups were sacrifised at regular time intervals (*i.e.*

during different phases of tail regeneration) and liver, thigh muscle, amputated limb stump and tail regenerate were taken for estimation of total protein content by the method of Lowry et al. (1950).

RESULTS

Subsequent to tail autotomy as well limb amputation, hepatic protein content depicted gradual depletion lasting upto 25 days. Since then, there was a tendency for general recovery of the hepatic protein content, though even on 60th day when tail regenerate is more or less fully grown the levels were subnormal. Though there was a parallelism in the pattern of protein depletion, that during post-limb amputation was noticeably more pronounced than that during post-tail autotomy. Similarly, the thigh muscle protein content too tended to show a close parallelism with gradual depletion during the first 10 days and sharper decline during the next 15 days, with the resultant attainment of lowest level on the 25th day. Thereafter, the muscle protein content showed a gradual increase. As far as the local protein contents were concerned, the tail protein content increased by the 3rd day post-autotomy and fell to normal level by the 7th day. Normal to slightly above normal levels of protein content were thereafter maintained till about

Table 1. Quantitative alterations in the tissue protein content (mg/100 mg fresh tissue) during tail regeneration in *H. flaviviridis*.

Periods of regeneration in days	N	1	2	3	5	7	10	20	25	40	60
Liver	21.871	24.992	26.552	27.139	24:7519	22.1784	19.619	16.643	17.784	17.314	20.371
	±4.795	±2.086	±1.87	±5.784	±1.189	±3.04	±3.61	±2.32	±3.147	±1.33	±0.97
							0.0025*				
Muscle	23.9373	22.77	18.65	19.464	19.813	25.4938	18.4546	8.1546	9.9156	14.209	11.130
	±1.08	±3.17	±2.43	±3.24	±1.75	±4.84	±3.47	±1.68	±2.32	±1.01	±1.39
Tail	7.014	-	-	10.435	8.283	7.364	8.233	8.152	6.715	9.23	8.936
	±1.203	-	-	±2.085	±1.87	±0.98	±2.53	±1.584	±0.945	±2.124	±1.43
				0.001*						0.001*	

± S. D.

* P value

Table 2. Quantitative alterations in the tissue protein content (mg/100 mg fresh tissue) post-limb amputation in <u>H. flaviviridis</u>.

Periods of regeneration in days	N	1	2	3	5	7	10	20	25	40	60
Liver	21.871 ±4.795	17.5753 ±0.666 0.01*	22.193 ±2.021	17.384 ±2.56	22.85 ±3.43	21.92 ±4.33	15.994 ±3.34	14.167 ±2.67	12.844 ±1.654	14.646 ±2.587	18.946 ±2.0
Muscle	23.9373 ±1.08	17.9408 ±1.68 0.0005*	22.13 ±3.57	18.135 ±1.49	19.885 ±2.01	16.5021 ±2.187 0.001*	17.9316 ±1.96	8.087 ±0.48	9.607 ±1.42	15.0193 ±3.53	10.777 ±2.062
Limb	9.922 ±2.083	- + -	- -	14.737 ±3.032	13.824 ±1.893	12.241 ±0.973 0.01*	16.062 ±4.107 0.0005*	9.436 ±0.082	6.088 ±0.763	6.375 ±1.147	11.515 ±2.725

± S. D.

* P value

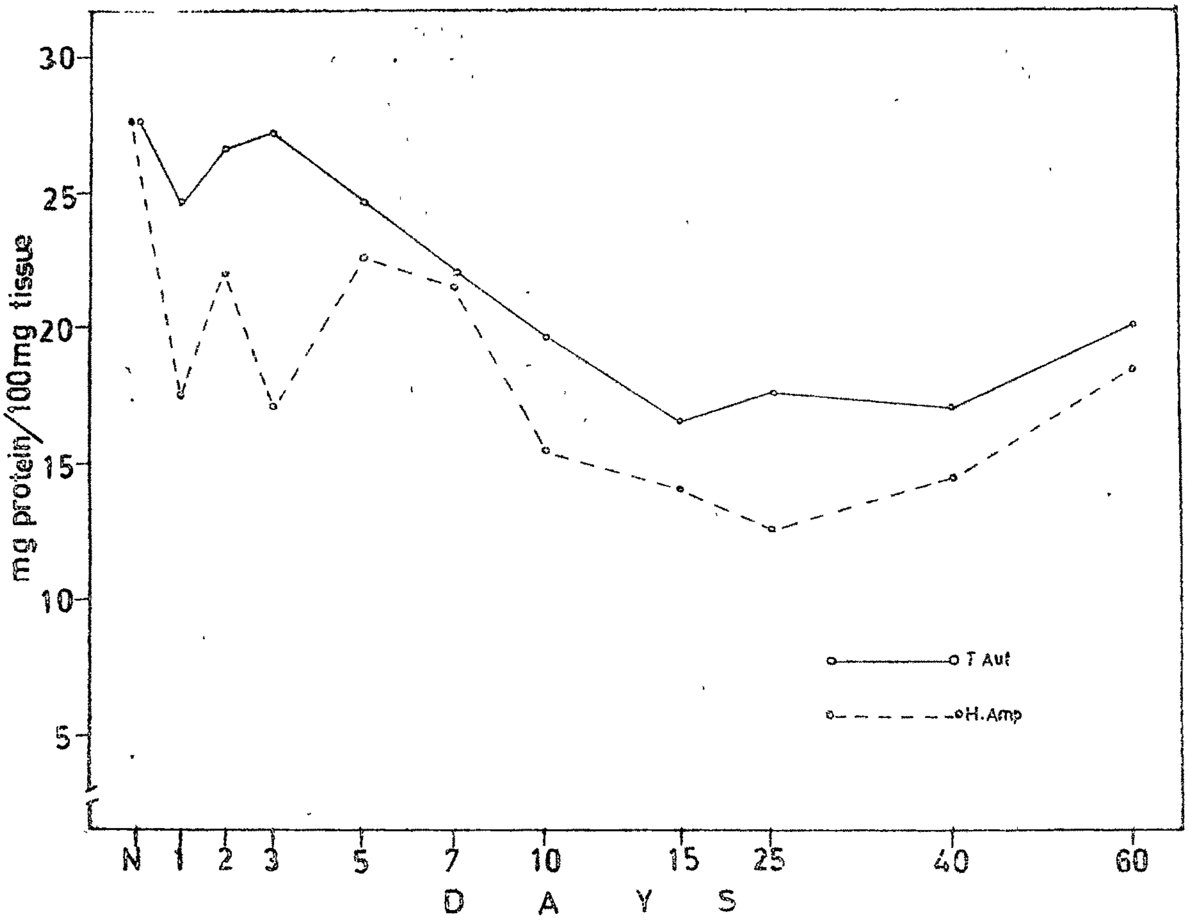

Fig. 1 : Graphic representation of the comparative levels of liver protein post-limb amputation and post-caudal autotomy in H. *flaviviridis*

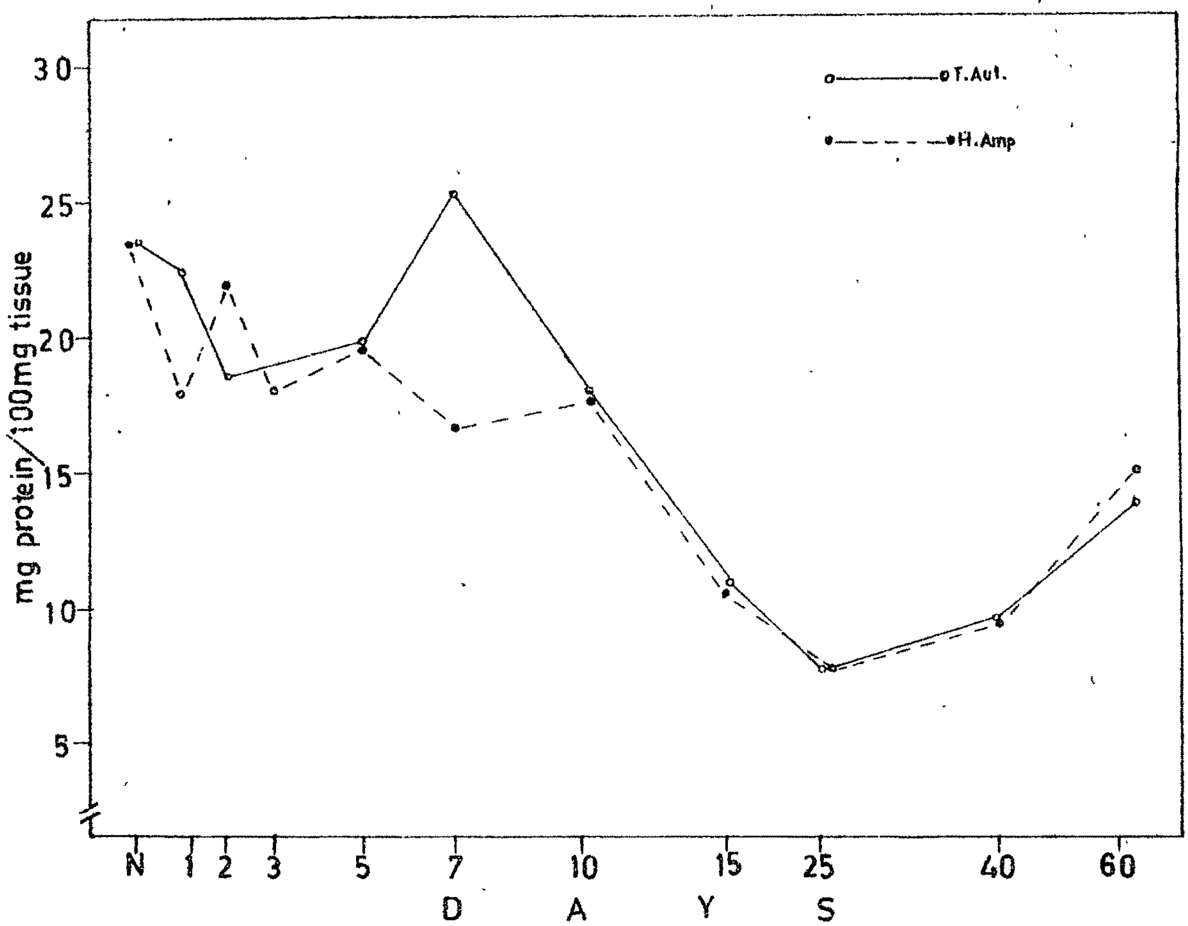

Fig. 2 : Graphic representation of the comparative levels of muscle protein post-limb amputation and post-caudal autotomy in H. *flaviviridis*

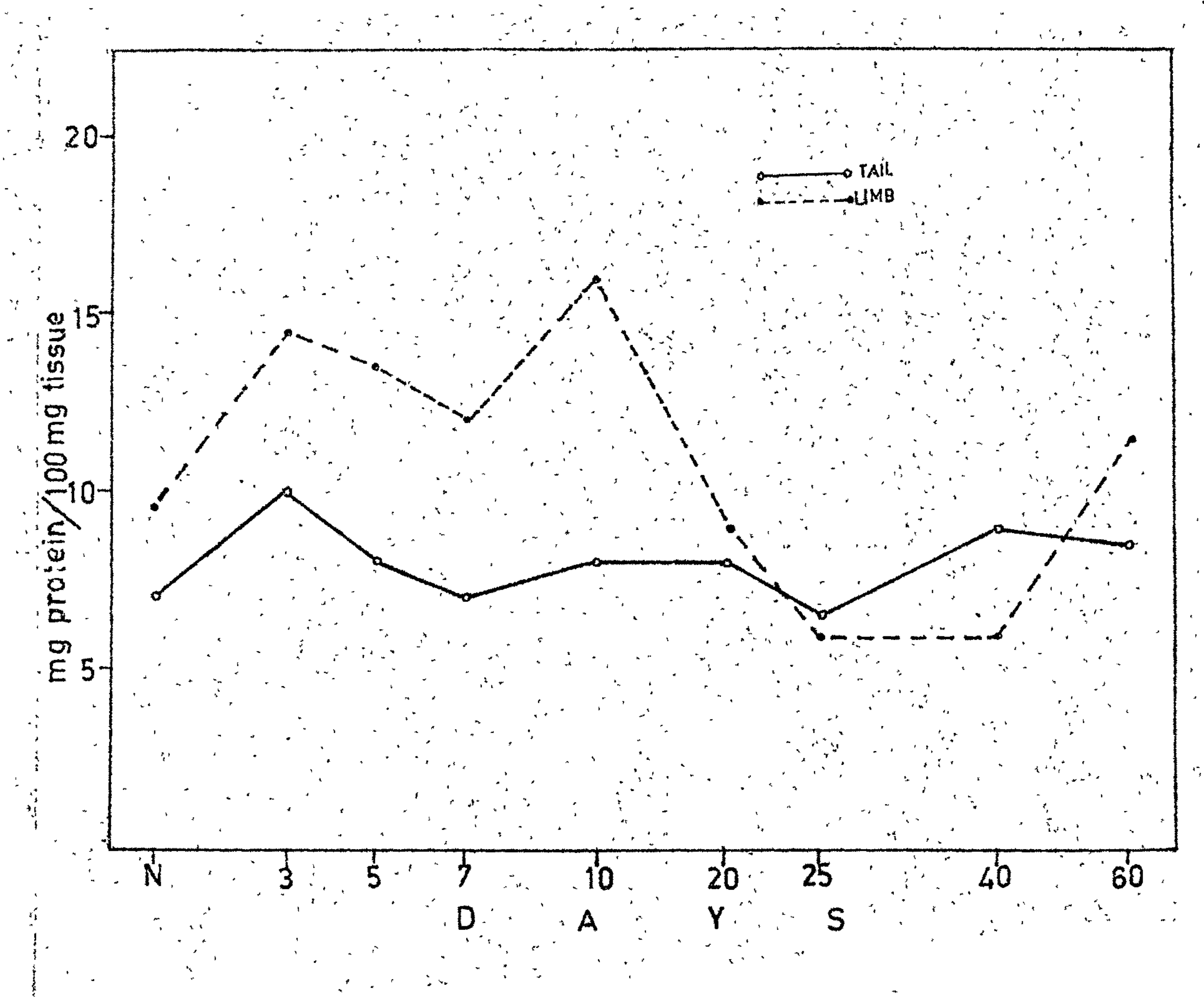

Fig. 3 : Graphic representation of the comparative levels of protein in the regenerate and limb stump post-limb amputation and post-caudal autotomy.

the 25th day of tail regeneration. This was followed by second phase of increase during the 25th and 40th days followed by gradual decrease towards the normal. In contrast, the amputated limb stump protein content showed an initial increase on the first three days with a slight fall during 3rd to 7th days, which again increased to the maximal level on the 10th day. Since then there was a steep fall till a sub-normal level was attained on the 25th day from which the level rose upto the pre-amputation level by the 60th day (Tables 1 and 2; Figures 1 and 2).

DISCUSSION

Protein synthesis constitutes one of the most basic features of cellular metabolism. Wound healing and regeneration both have the common features like synthesis of new structural elements for the wound closure or for regeneration respectively. Earlier works from this laboratory cited above have shown the involvement of nucleic acids and protein during regeneration and also mere wound healing post-caudal amputation in a non-regenerating lizard (Shah et al., 1980). The study of Shah et al. (1980) on systemic alterations in nucleic acids and protein contents post-caudal amputation in *Calotes versicolar*, a lizard with no regenerative potential, had reported a dissimilar pattern from that occurring in Mabuya, a lizard with tail regenerative potential. The present study is, however, a comparative account of changes occurring in the same lizard, H. flaviviridis. Systemic protein responses recorded in the present study during tail regeneration appears to be distant from those reported for Mabuya carinata (Ramachandran et al., 1980). The positive protein balances recorded in Mabuya as opposed to the herein noted negative nitrogen balance in Hemidactylus tend to underscore the differential metabolic adaptation characteristic of these two lizards. In the absence of protein depletion (Ramachandran et al., 1980), significant depletion of muscle glycogen coupled with increased transaminase activity were suggested to be the prime source of amino acids for meeting the requirements of regeneration in Mabuya (Shah et al., 1982; Ramachandran et al., 1982). In contradistinction, with no contribution from muscle glycogen (Chapter 1), the significant loss of systemic proteins (Fig. 1) definitely indicate the participation of systemic sources of proteins in Hemidactylus for the local requirements at the site of regeneration. Pertinently, Steele (1975) has reported that increased amino acid utilization in gluconeogenesis leads to increased glycogen deposition and overall negative nitrogen balance. Again, Ballard (1980) has reported that increased responsiveness to insulin can also modulate hepatic protein synthesis or degradation. Insulin dependancy of Hemidactylus may be relevant in this respect. Moreover, glucocorticoid has been shown to inhibit protein synthesis and increase protein degradation in liver and muscle (Chertow et al., 1973; Kim and Kim, 1975; Wool and Weishelbanin, 1960; Golgerg, 1969, 1975; Young, 1970; Tomas et al., 1979).

Looking at the responses obtained post-limb amputation in Hemidactylus, it becomes obvious that the changes in hepatic and muscle protein contents are strikingly identical and parallel to those observed during post-tail autotomy, thus signifying a similar systemic response to loss of any body extremity, may it be limb or tail in *H. flaviviridis*. Local changes indicate, however, a more optional protein metabolism in case of post-caudal autotomy depicting maintainence of normal to slightly above normal levels of protein all throughout regeneration, and hyper-sensitive less coordinated alterations post-limb amputation marked by pronounced increase in protein content during the first 10 days and sharp depletion to a sub-normal level from the 10th to 25th days. Apparently, as in the case of carbohydrate metabolism (Chapter 1), protein metabolism also depicts similarity in response between post-caudal autotomy and post-limb amputation systemically, while locally, once again, amputation of an extremity which does not possess the ability to regenerate, fails to regulate the protein turnover to adaptive optimum levels. Thus, inability of the local site to adapt and adjust to protein turnover to a pattern characteristic of regeneration (as in tail) may probably be implicated in the cause effect relationship responsible for the lack of regenerative ability in some extremities (limbs) of the lizard body.