CHAPTER 4

COMPARATIVE EVALUATION OF QUANTITATIVE ALTERATIONS IN HEPATIC AND MUSCLE PHOSPHATASES POST-CAUDAL AUTOTOMY AND POST-LIMB AMPUTATION IN *HEMIDACTYLUS FLAVIVIRIDIS*

Life depends upon the complex network of chemical reactions that are brought about by enzymes or rather multi-enzyme systems. These multienzyme systems and the mechanism of their control are genetically based. Activation and repression may be considered the basis of regeneration. Phosphatases are certain enzymes which catalyse the splitting of the phosphoric acid from certain monophosphoric esters, a reaction of considerable importance in several body processes. Acid phosphatase is supposed to be involved in a variety of cellular activities such as cellular phagocytosis (Klockars and Wegelius, 1969) dissolution of tissue components (Weber and Nichus, 1961; Cohen, 1970), protein synthesis (Pearse, 1968), differentiation (Ghiretti, 1950), transport (Friede, 1964), absorption and phosphorylation (Steetan, 1964). Alkaline phosphatase is demonstrated to be involved in differentiation, formation of fibrous proteins, calcification of ground substances (Simkiss, 1964), carbohydrate metabolism (Rosenthal *et al.*, 1960), phosphate transfer in DNA metabolism (Rogers, 1960) and passage of

metabolites across cell membrane (Simkiss, 1964). Acid and alkaline phosphatases have been considered to be associated with the regenerative processes in amphibians (Schmidt, 1968) and reptiles (Shah and Chakko, 1966; 1967; Radhakrishnan, 1972). An attempt is made, here, to study the response of hepatic and muscle phosphatases (acid and alkaline) as a part of the systemic response during tail regeneration in Hemidactylus flaviviridis. The alterations so obtained post-caudal autotomy have been further compared with the responses that occur post-limb amputation in the same lizard, so as to ascertain whether the same systemic responses are initiated even after the partial loss of a body extremity (limb) incapable of undergoing regeneration.

MATERIALS AND METHODS

Adult H. flaviviridis collected from Baroda were kept in the laboratory on a diet of insects for a fortnight. The animals were then divided into two groups. One group of animals was subjected to caudal autotomy and the other to fore-limb amputation. At successive intervals (3rd, 5th, 7th, 10th, 20th, 25th, 40th, and 60th days) the animals were sacrifised. Liver and muscle were collected, weighed and homogenised in cold distilled water. The hómogenates were utilized for the estimation of acid and alkaline phosphatase

employing the methods described in Sigma Technical Bulletin No. 104 using p-NP as the substrate.

RESULTS

The changes in the activity levels of the two phosphatases in liver and muscle under both the experimental conditions could be generalised as follows : 1. Hepatic acid phosphatase tended to be above normal under both the conditions all throughout, while, hepatic alkaline phosphatase tended to be below normal all throughout. 2. The pattern of changes of hepatic acid and alkaline phosphatase tended to be exactly parallel during tail regeneration, with three peaks, corresponding to 5th, 10th, and 25th days post-autotomy. 3. Muscle acid and alkaline phosphatases after limb amputation showed a more or less parallel set of changes with an initial decrease to sub-normal levels by 3rd day (for alkaline phosphatase) and 5th day (for acid phosphatase), and increased to above normal levels by 7th and 10th days respectively. This was followed by decrease to sub-normal level by 20th day in both cases, and then a recovery towards normal levels thereafter. 4. Whereas hepatic acid phosphatase after limb amputation showed two peaks of activity, once during 5th and once during the 20th day post-amputation,

Table 1. Comparative levels of alkaline phosphatase (μ mole p-nitrophenol released/mg protein/ 30 minute) post-limb amputation and post-caudal autotomy in H. flaviviridis.

Periods of regeneration in days	N	3	5	7	10	20	25	40	60
Post-caudal autotomy									
Liver	0.3387 ±0.05388	0.5343 ±0.1998	0.8917 ±0.2459	0.5484 ±0.1366	0.833 ±0.1743	0.3969 ±0.0596	0.7808 ±0.1393	0.399 ±0.05926	0.3086 ±0.2288
Muscle	0.03845 ±0.0083	0.01837 ±0.003501	0.0203 ±0.01799	0.02706 ±0.01054	0.0327 ±0.04509	0.0462 ±0.00306	0.03527 ±0.001717	0.0293 ±0.01102	0.02088 ±0.00981
Post-limb amputation									
Muscle	0.03845 ±0.00830	0.01617 ±0.00526	0.03125 ±0.00234	0.04739 ±0.00512	0.0425 ±0.00524	0.01748 ±0.00576	0.02241 ±0.00311	0.0246 ±0.00247	0.0304 ±0.001397
Liver	0.3387 ±0.05388	0.4729 ±0.02295	0.4753 ±0.01056	0.5749 ±0.02154	0.8348 ±0.03146	0.5177 ±0.01860	0.309 ±0.02772	0.483 ±0.05798	0.3614 ±0.01258

± S. D.

Table 2. Comparative levels of acid phosphatase (μ mole p-nitrophenol released/mg protein/30 minute) post-limb amputation and post-caudal autotomy in H. flaviviridis.

Periods of regeneration in days	N	3	5	7	10	20	25	40	60
Caudal autotomy									
Liver	0.6249 ±0.1230	0.7428 ±0.1853	0.9961 ±0.299	0.7552 ±0.1054	0.8895 ±0.2378	0.5450 ±0.05982	0.8685 ±0.11265	0.6214 ±0.1159	0.6647 ±0.228
Muscle	0.1810 ±0.0830	0.1847 ±0.03501	0.1776 ±0.03903	0.1359 ±0.0451	0.1799 ±0.04510	0.0895 ±0.02137	0.1354 ±0.0292	0.06436 ±0.03067	0.08854 ±0.0177
Limb amputation									
Liver	0.6249 ±0.1230	0.6217 ±0.1853	0.8018 ±0.04101	0.6812 ±0.01548	0.6713 ±0.01052	0.8786 ±0.07137	0.766 ±0.1565	0.7685 ±0.1605	0.771 ±0.1557
Muscle	0.1810 ±0.0830	0.1548 ±0.0236	0.0542 ±0.01738	0.122 ±0.01928	0.2146 ±0.0212	0.1277 ±0.05468	0.1296 ±0.0109	0.1117 ±0.02128	0.1492 ±0.0093

± S. D.

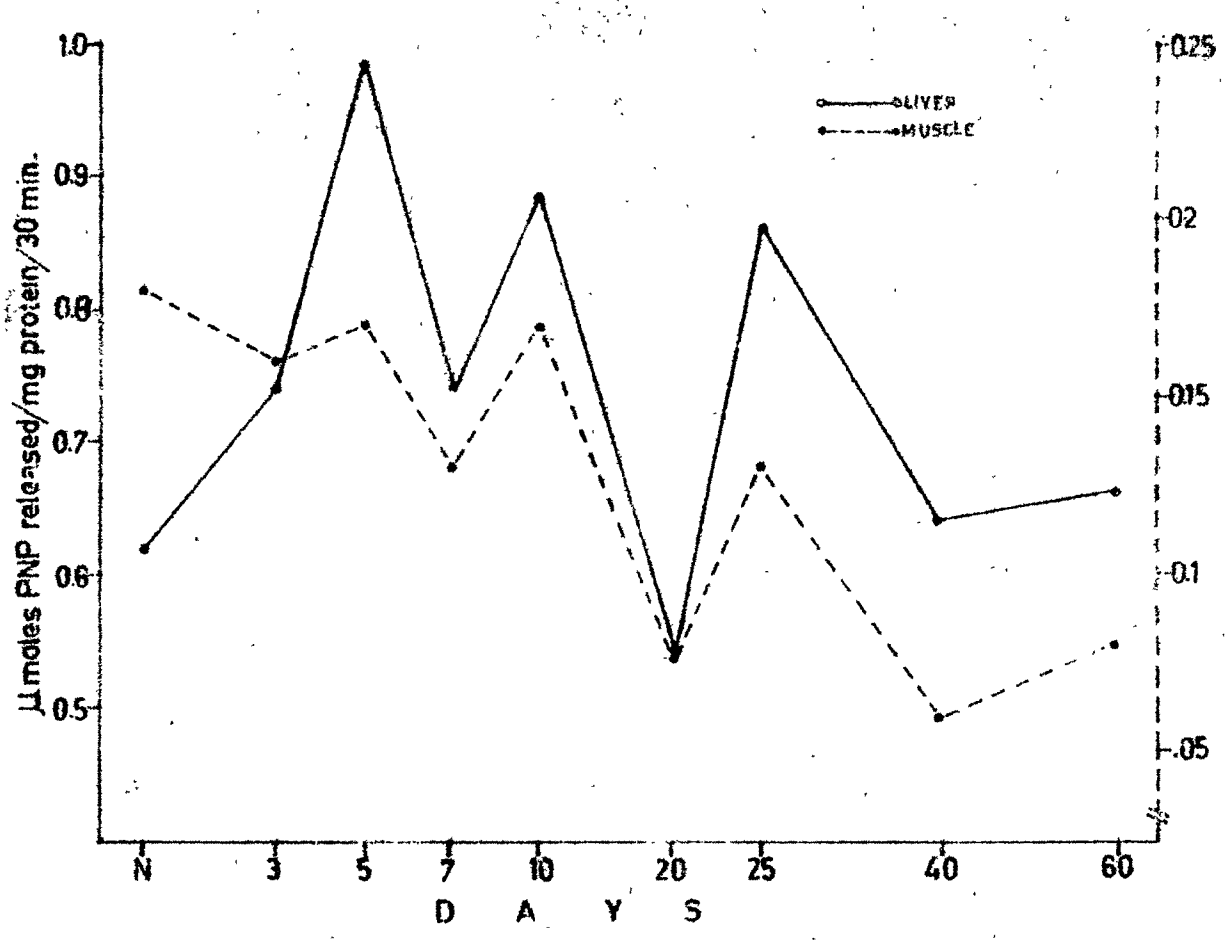

Fig. 1 : Graphic representation of the levels of acid phosphatase in the liver and muscle during tail regeneration in the lizard, H. flaviviridis

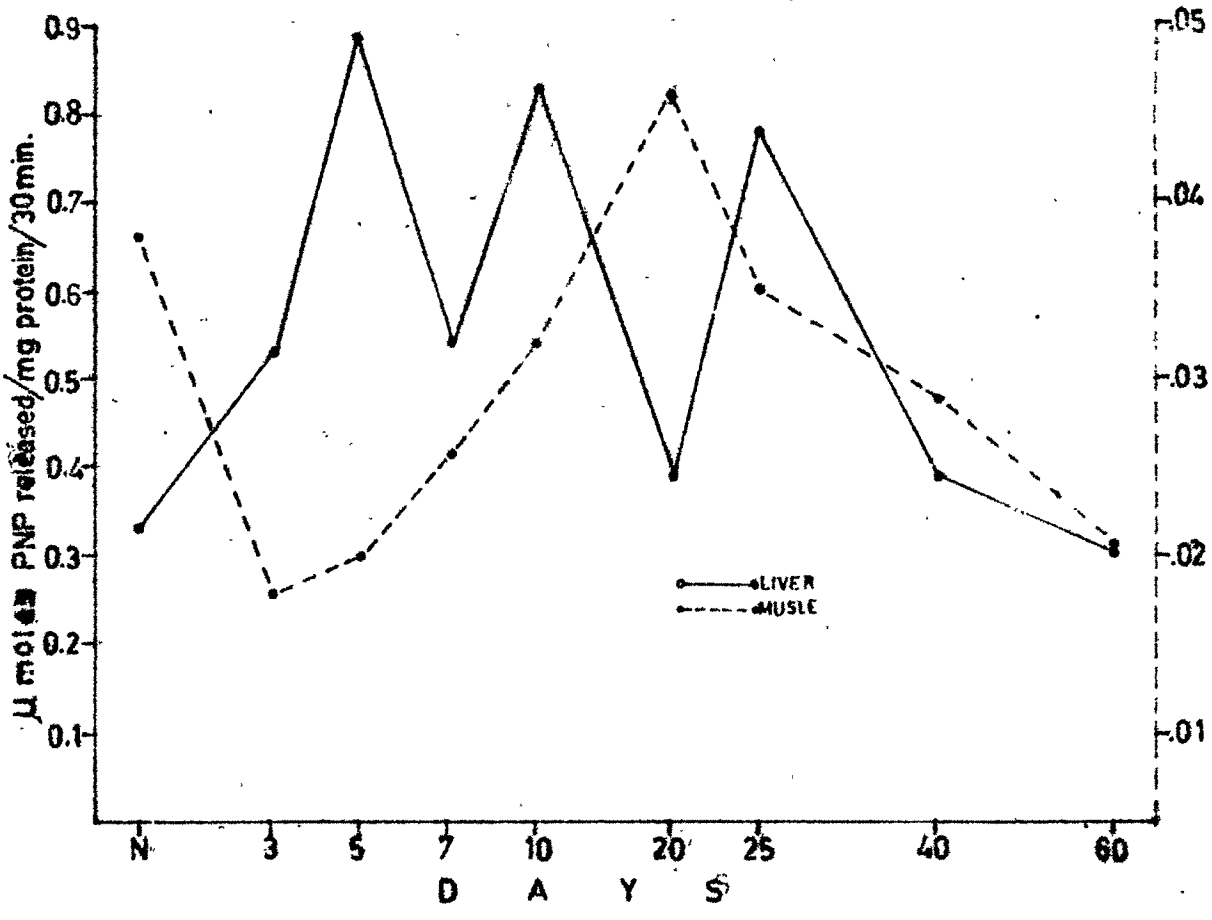

Fig. 2 : Graphic representation of the levels of alkaline phosphatase in the liver and muscle during tail regeneration in the lizard, H. flaviviridis

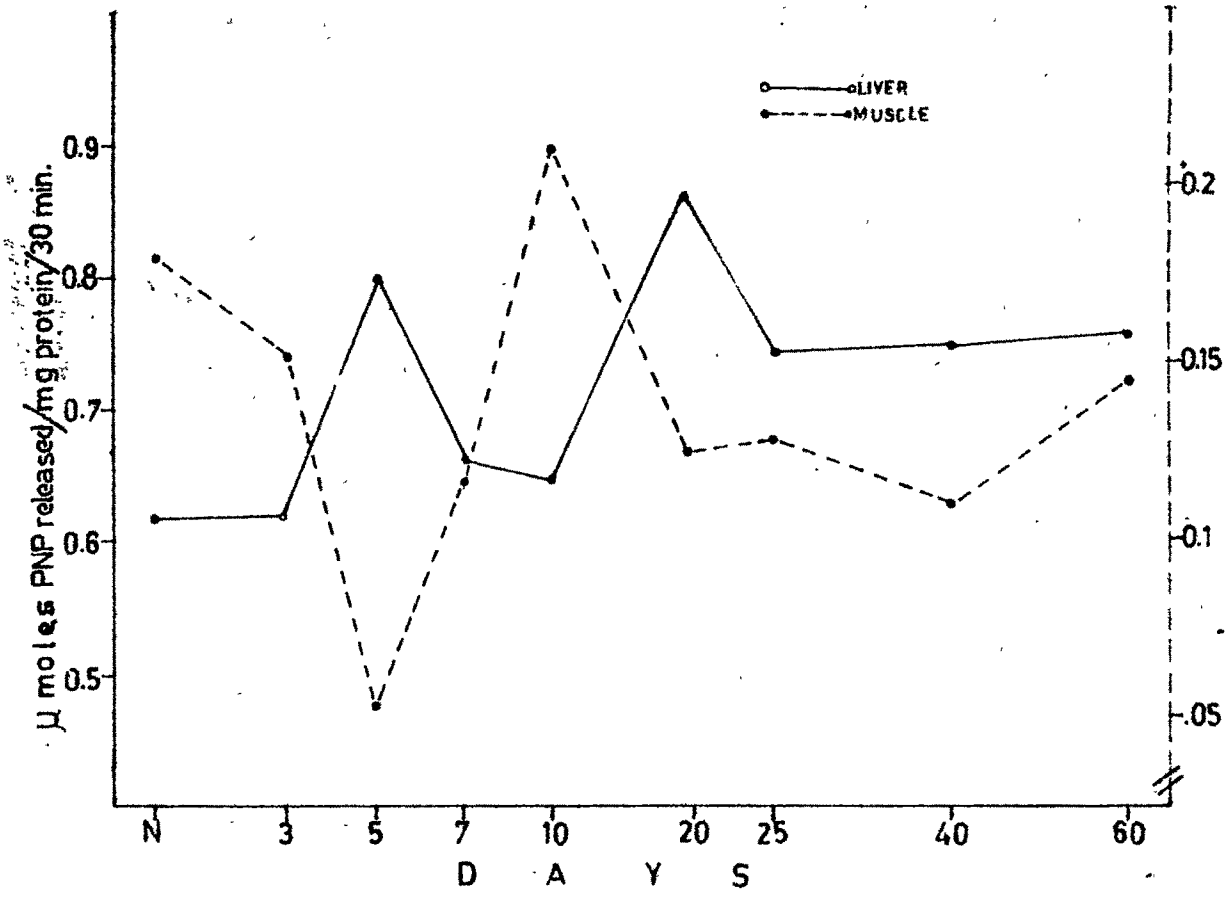

Fig. 3 : Graphic representation of the levels of acid phosphatase in the liver and muscle post-limb amputation in the lizard, H. flaviviridis

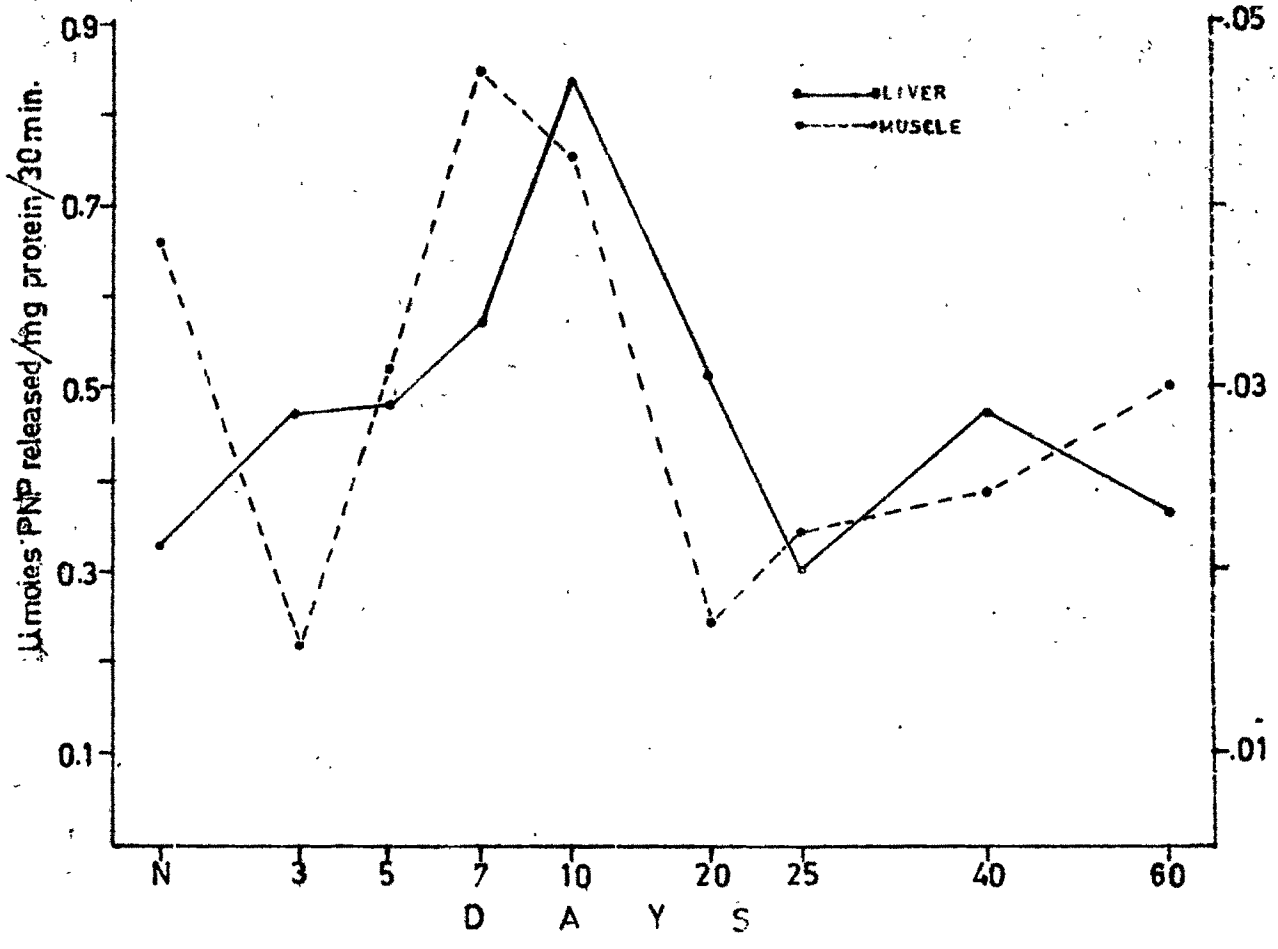

Fig. 4 : Graphic representation of the levels of alkaline phosphatase in the liver and muscle post-limb amputation in H. flaviviridis.

hepatic alkaline phosphatase showed a pronounced peak on the 10th day and a small peak on the 40th day. 5. Muscle acid phosphatase showed a more or less linear fall in its activity (with slight zig zag pattern in between) reaching the lowest sub-normal level by the 40th day. Muscle alkaline phosphatase dropped sharply to sub-normal level by 3rd day followed by an increased above normal level by 20th day and a further gradual decrease to below normal level lasting upto the 60th day.

DISCUSSION

Phase specific *in loco* involvement of phosphatases during tail regeneration in *H. flaviviridis* and *M. carinata* have been already documented (Shah and Chakko, 1966, 1967; Radhakrishnan, 1972). However, the systemic alterations (known to affect many physiologic parameters) of phosphatase have not been looked into, and the present evaluation is pertinent in this context. Results obtained herein indicate tremendous activation of both the phosphatases in liver; in contrast, muscle phosphatases have tended to be decreased during regeneration. In this light of the purported active participation of liver in lacertilian tail regeneration (previous publications from this laboratory), the increment in hepatic phosphatases is self explanatory as these enzymes

are correlated with many physiological activities. On a comparative basis, alkaline phosphatase appears to be more involved in the physiological alterations occurring in liver.

The results recorded (Figs. 3 and 4; Table 1-2) also indicate a more or less similar systemic response even post-limb amputation, though not followed by regeneration. There are, however, less pronounced effects on qualitative modulations involved. Moreover, the immediate post-limb amputation fall in thigh muscle phosphatase is more prominent as compared to that post-caudal autotomy. Again, the elevation in hepatic alkaline phosphatase activity appears to be gradual and slow to attain during limb amputation as compared to caudal autotomy, whence it was sudden and swift. Apart from these minor differences, the general trend of systemic responses for phosphatases were more or less identical in the two conditions viz. limb amputation and tail autotomy. It may be concluded from the present that though there is a general permissiveness pervading the body for phosphatase activity post-limb amputation and post-caudal autotomy, the phase specific modulations are dependent on regeneration specific informational inputs from the local site.

CHAPTER 5

IN LOCO AND SYSTEMIC RESPONSES TO SIMULTANEOUS CAUDAL AUTOTOMY AND LIMB AMPUTATION IN HEMIDACTYLUS FLAVIVIRIDIS

Wound closure is probably one of the most investigated aspects of medical research (Russel and Billingham, 1962). Closure is achieved by a combination of two processes, contraction and epithelialization. These two processes appear to be independent of each other (John, 1981). Their relative contribution to final closure are dependent on a number of factors including the site and size of the wound (Carrel and Hartman, 1916; Arey, 1936; Van den Brenk, 1956; Billinghan and Russel, 1956). During regeneration, incomplete wound healing is followed by the growth of the lost part. Significance of local surface potential and surface area for regeneration is still argumented. Apart from the mechanics of wound healing, the loss of tail in lizards, is marked by many favourable local dispositions and adaptive systemic responses as have been enlightened by the many previous publications from this laboratory. Further, the previous chapters (1-4) have tried to analyse the systemic response to amputation of fore-limb in Hemidactylus flaviviridis as a possible control to those that occur due to caudal autotomy in the same lizard. The

results obtained indicated a fundamental similarity in systemic responses to both the situations, thus giving rise to a dictum that in an animal even with partial regenerative potency, the systemic responses elicited remain essentially the same whenever a body extremity either with regenerative potency or otherwise is lost. As a follow up, the ability of caudal autotomy to evoke regenerative potency on simultaneous fore-limb amputation or even the possibility of simultaneous limb amputation to retard tail regeneration are attempted in the present study together with evaluation of various parameters already identified as part of the collective systemic response during regeneration in lizards.

MATERIALS AND METHODS

Adult H. flaviviridis collected from the local area were kept on a standard diet of insects for a fortnight. The animals were amputated (Fore-limb) and autotomized (tail) simultaneously to see the resulting changes. Along with morphological observations on local events, various systemic parameters like levels of protein, glycogen, glucose, ascorbic acid and activities of acid and alkaline phosphatase were also evaluated according to the respective procedures mentioned in the previous chapters.

RESULTS

Morphological observations on the local site indicated neither any evokement of the regenerative potency in the amputated limb nor any retardation in the regenerative growth of the tail regenerate, as depicted by the normal rate of regeneration [illegible]. Simultaneous autotomy and amputation showed a decrease in the blood glucose level till 3rd day, followed by a gradual increase during 5th and 7th days. On the 10th day, it attained the minimum level, followed by a slight increased level on 15th day. All throughout, a sub-normal level was, however, noticed.

Glycogen content of the liver, muscle, and tail showed a more or less similar pattern till the 15th day with an initial depletion lasting upto the 5th day and an increase on the 7th day. Liver and muscle showed an increased glycogen content on the 1st day. Muscle glycogen attained the maximum peak on the 10th day, and a depletion on the 15th day whereby it attained a sub-normal level. Liver showed glycogen depletion from the 7th day onwards, though it remained in an above normal level. Tail glycogen showed an increase on 7th, 10th and 15th days to attain the normal level on 15th day. Limb glycogen showed an initial depletion till the 3rd day followed by attainment of a

Table 1. Alterations in the levels of blood glucose (mg/100 ml blood) and tissue glycogen content (mg/100 mg fresh tissue) under simultaneous limb amputation and caudal autotomy in H. flaviviridis.

Periods of regeneration in days	N	1	2	3	5	7	10	15
Blood glucose	114.00 ± 8.24	106.23 ±12.04	78.4272 ±10:3 0.005*	72.36 ± 5.88	82.44 ±11.07	95.43 ± 9.99	67.211 ±12.53	71.98 ± 8.86
Liver	0.455 ± 0.104	1.22 ± 0.196	0.2912 ± 0.1256	0.1876 ± 0.1015	0.2146 ± 0.0106	1.4626 ± 0.2023	1.1906 ± 0.2846	0.762 ± 0.1623
Muscle	0.0922 ± 0.0175	0.2370 ± 0.0997	0.0547 ± 0.001424	0.038 ± 0.0015	0.02028 ± 0.001729	0.1547 ± 0.0037	0.2284 ± 0.012248	0.0572 ± 0.0042
Tail	0.3066 ± 0.084	0.07685 ± 0.0297	0.0359 ± 0.003057	0.0421 ± 0.01042	0.0475 ±0.00235	0.1795 ± 0.01222	0.2809 ± 0.0488	0.302 ± 0.1014
Limb	0.1602 ± 0.008	0.12905 ± 0.00649	0.158 ± 0.00632	0.164 ± 0.02158	0.1507 ±0.0154	0.1827 ± 0.01534	0.2635 ± 0.0181	0.2612 ± 0.011

± S. D.

* P value

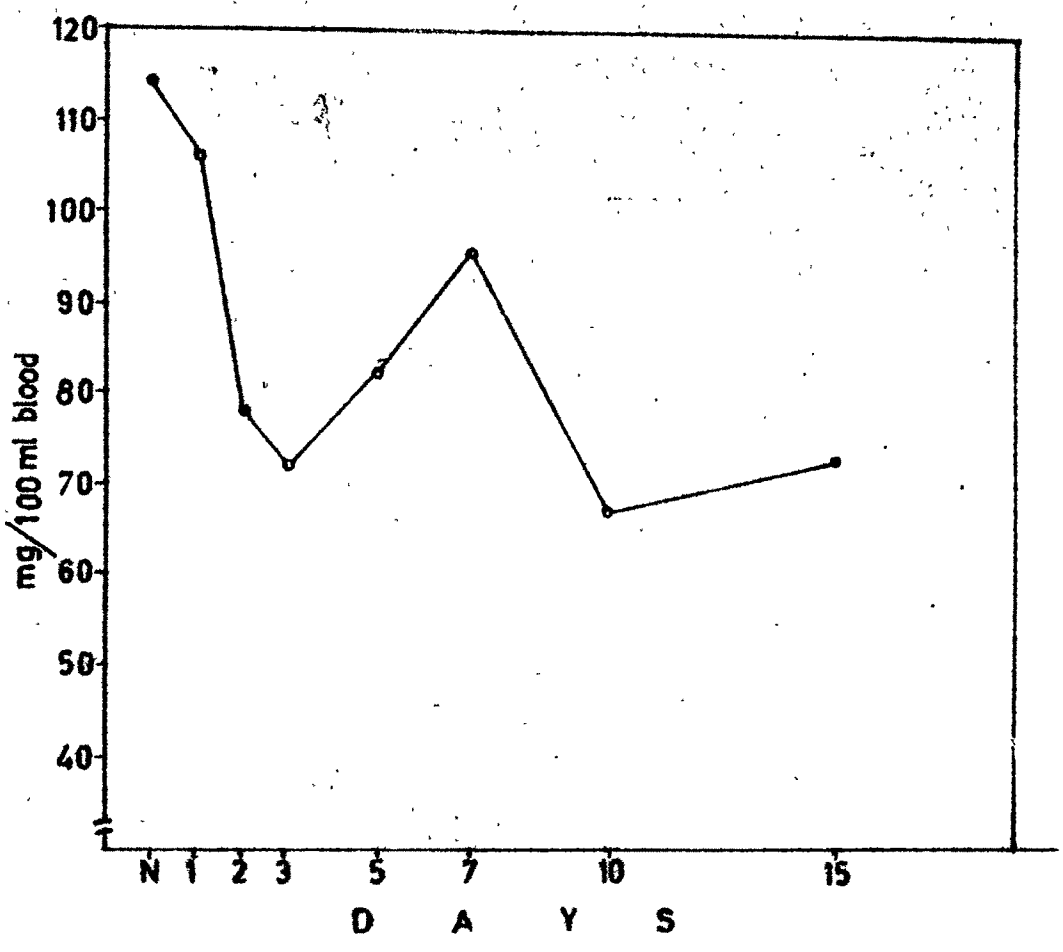

Fig. 1 : Graphic representation of the levels of blood glucose under simultaneous limb amputation and tail autotomy in the lizard, H. flaviviridis

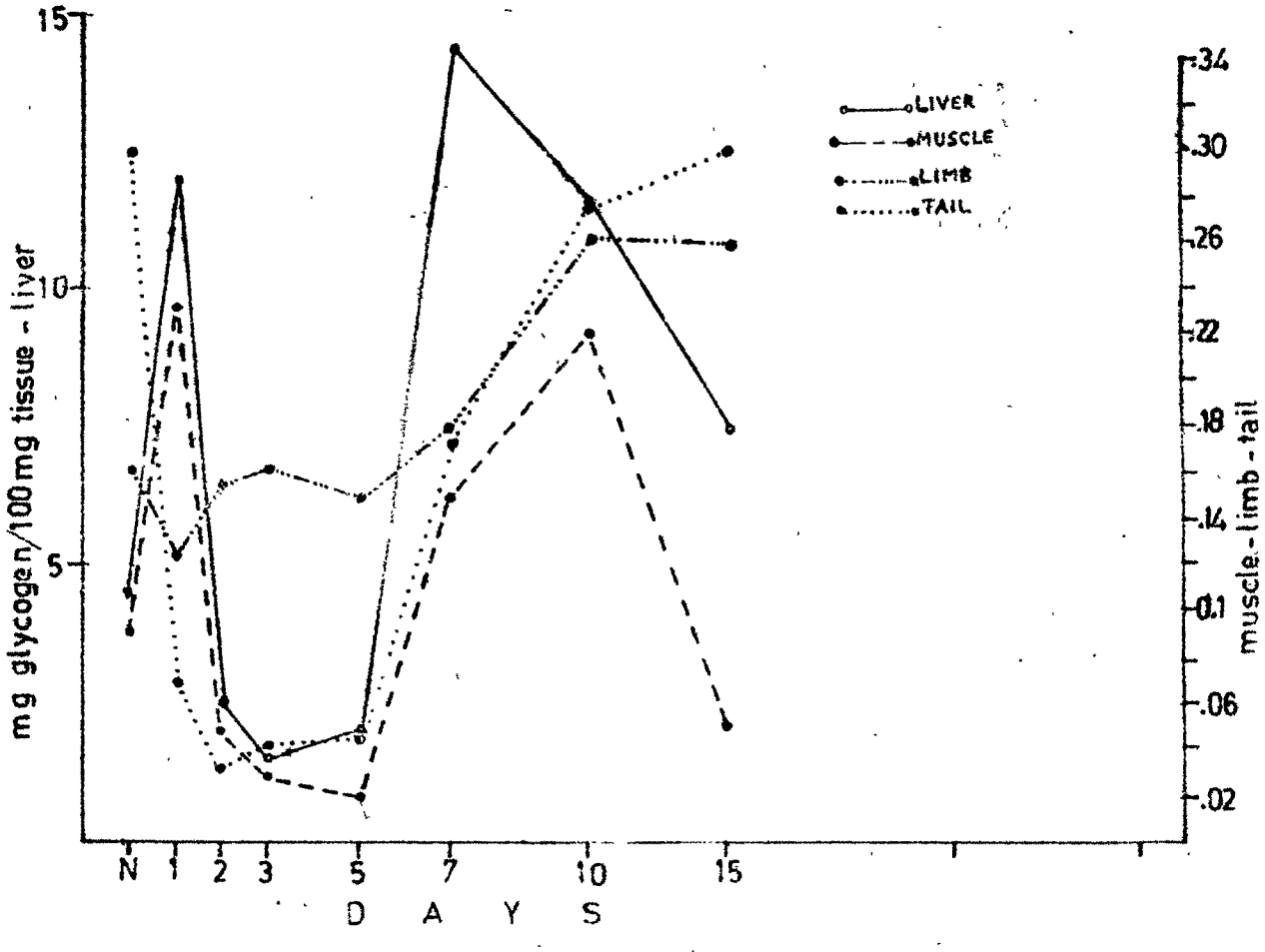

Fig. 2 : Graphic representation of the levels of tissue glycogen under simultaneous limb amputation and tail autotomy in H. flaviviridis.

Table 2. Quantitative alterations in tissue protein content (mg/100 mg) under simultaneous limb amputation and caudal autotomy in H. flaviviridis.

Periods of regeneration in days	N	1	2	3	5	7	10	15
Liver	27.871 ±4.87	12.1005 ±2.2267 0.0005*	17.3 ±1.1879 0.005*	14.27 ±0.5197	10.2145 ±0.5892	17.533 ±3.18339 0.0005*	15.621 ±1.12995	17.844 ±1.325
Muscle	23.9373 ±1.08	17.426 ±1.8552 0.0005*	11.243 ±0.9948	8.254 ±0.7148	4.749 ±0.8215	11.2215 ±0.9779 0.005*	12.8405 ±1.8236	11.1753 ±1.623
Tail	7.014 ±1.203	6.363 ±1.476 0.01*	8.2184 ±2.379	7.285 ±1.482	4.6436 ±2.1614	7.362 ±2.006 0.01*	7.23 ±0.3549	9.38 ±0.5821
Limb	9.922 ±2.083	15.699 ±2.3032 0.0005*	6.445 ±1.2158	5.115 ±1.1812	4.07 ±1.6235	10.135 ±1.0623 0.0005*	10.358 ±2.764	10.536 ±1.9214

± S. D.

* P value

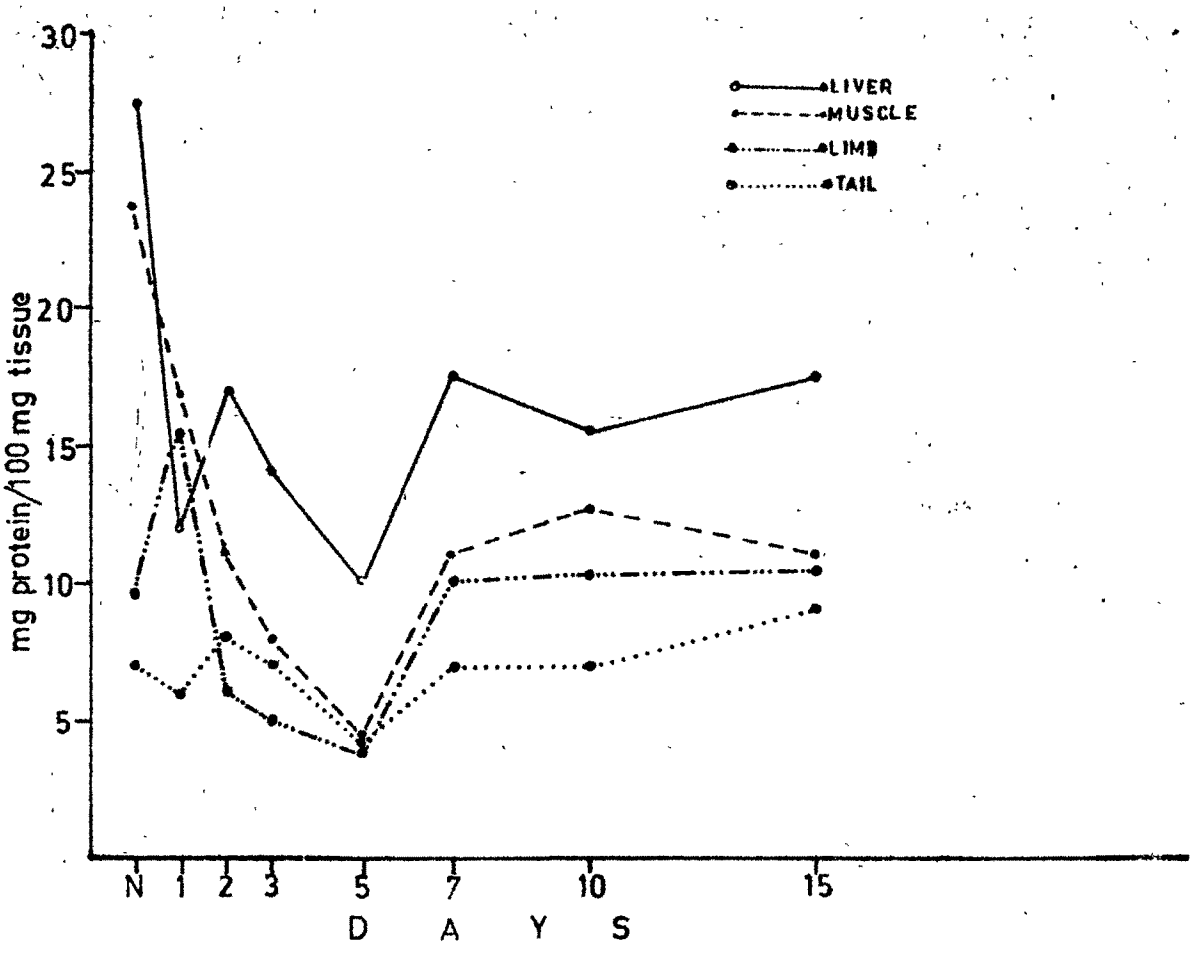

Fig. 3 : Graphic representation of the levels of protein in the liver, muscle, limb stump and the regenerate under simultaneous tail autotomy and limb amputation in the lizard, H. flaviviridis

normal level on the 5th day. Gradual increase was noticed, thereafter, till the 15th day.

Protein content of liver, muscle, tail and limb showed a more or less similar pattern of fluctuations with an initial depletion followed by a gradual increase. The content in all the tissues attained a minimum sub-normal level on the 5th day and an increased above normal level on the 7th day. Muscle and liver protein contents remained sub-normal level till the 15th day. Liver protein level differed from that of the muscle by showing an increase on the 3rd day; limb protein increased on the 1st day followed by a depletion till the 5th day. It increased thereafter and attained a more or less normal value by the 15th day. Tail protein showed slight depletion on the 1st day. Its level rose on 3rd day followed by a decrease on the 5th day. Gradually the level increased to an above normal level by the 15th day.

Ascorbic acid content of the limb and kidney showed a parallel pattern with a depletion on the 3rd day and an increase on the 5th day followed by a fall on the 7th day. An increased level was noticed in both the cases on the 10th and 15th days whereby the kidney ascorbic acid attained the normal level and limb AA content remained in a sub-normal

Table 3. Alterations in the levels of ascorbic acid (mg/100 gm tissue) under simultaneous limb amputation and caudal autotomy in H. flaviviridis.

Periods of regeneration in days	N	1	2	3	5	7	10	15
Liver	23.2 ±3.28	20.05 ±1.47 0.01*	14.9 ±1.94	10.1 ±1.82	8.5 ±1.02	17.9 ±2.453 0.0005*	23.45 ±1.421	13.4 ±0.901
Muscle	28.4 ±1.22	18.2 ±1.94 0.0005*	10.85 ±1.07	10.4 ±0.09	13.5 ±3.3	8.05 ±2.28	16.9 ±1.28	27.7 ±1.08
Tail	5.82 ±0.334	5.9 ±1.559	5.35 ±1.718 0.01*	5.0 ±1.01	5.65 ±1.23	7.7 ±1.32	10.2 ±0.09	17.1 ±2.1 0.0005*
Limb	25.7 ±1.218	11.9 ±3.3 0.0005*	11.0 ±3.18	9.0 ±2.628	12.8 ±2.44	9.75 ±2.55	10.7 ±1.623	15.14 ±2.67

± S. D.

* P value

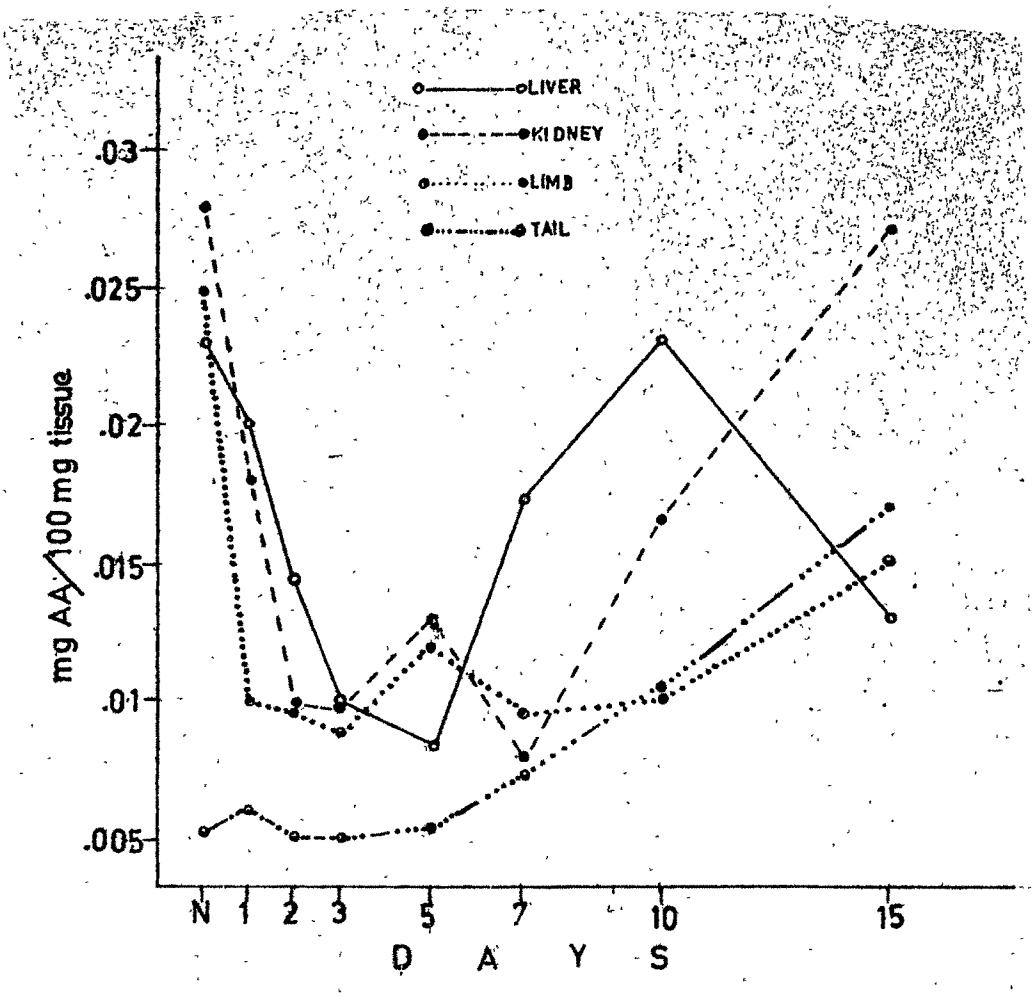

Fig. 4 : Graphic representation of the levels of tissue ascorbic acid (AA) under simultaneous limb amputation and tail autotomy in H. flaviviridis.

Table 4. Levels of alkaline and acid phosphatase (μ mole p-nitrophenol released/mg protein/ 30 minute) under simultaneous limb amputation and caudal autotomy in H. flaviviridis.

Periods of regeneration in days	N	3	5	7	10	15
Alkaline PO_4ase						
Liver	0.3387 ±0.0538	0.3915 ±0.02715	0.4245 ±0.09885	0.5010 ±0.03309	0.1144 ±0.02938	0.0950 ±0.00458
Muscle	0.03845 ±0.008305	0.0219 ±0.00621	0.0161 ±0.00417	0.0340 ±0.0011995	0.0485 ±0.00311	0.04405 ±0.00421
Acid PO_4ase						
Liver	0.6249 ±0.1230	0.5998 ±0.1197	0.6504 ±0.1012	0.6547 ±0.07021	0.440 ±0.02615	0.505 ±0.0420
Muscle	0.1810 ±0.0830	0.1516 ±0.06203	0.1752 ±0.0811	0.1875 ±0.01852	0.1205 ±0.02124	0.1329 ±0.01415

± S. D.

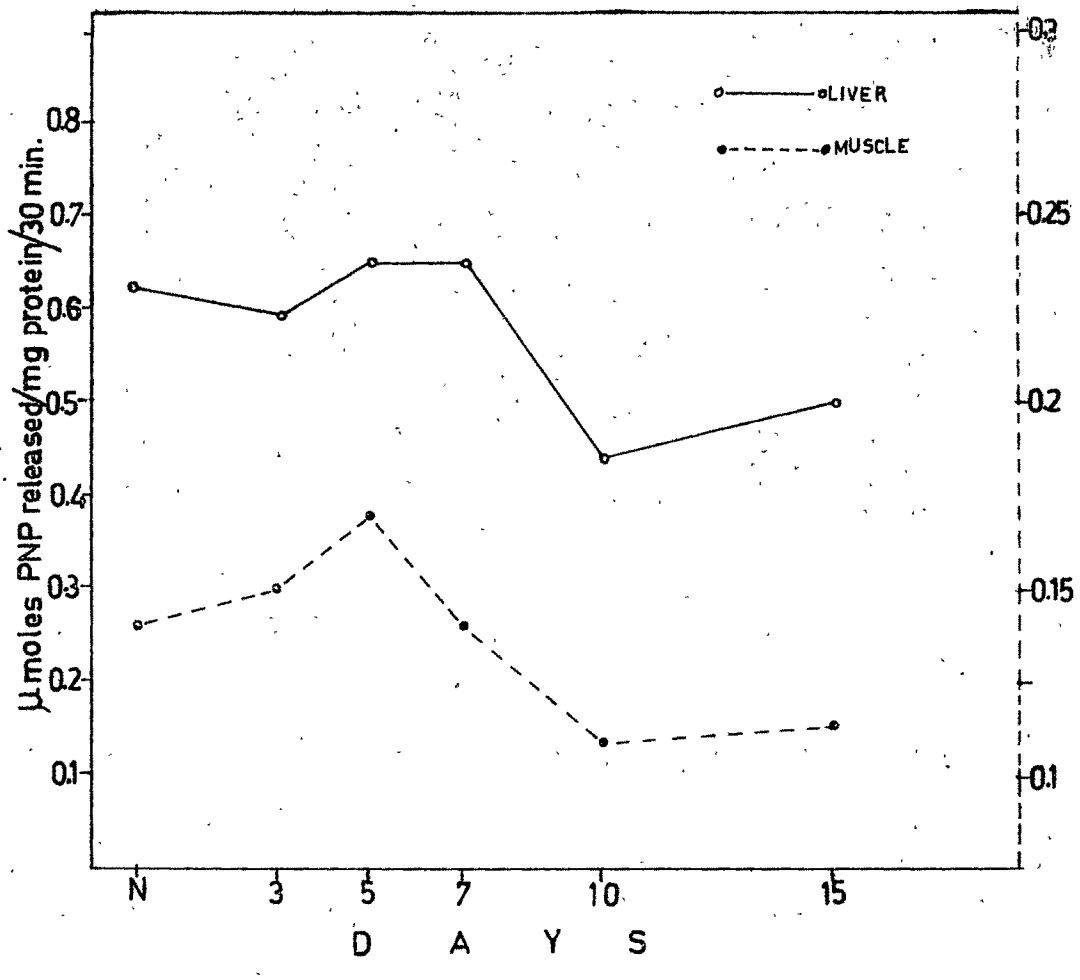

Fig. 5 : Graphic representation of the levels of acid phosphatase in the liver and muscles under simultaneous limb amputation and caudal autotomy in H. flaviviridis

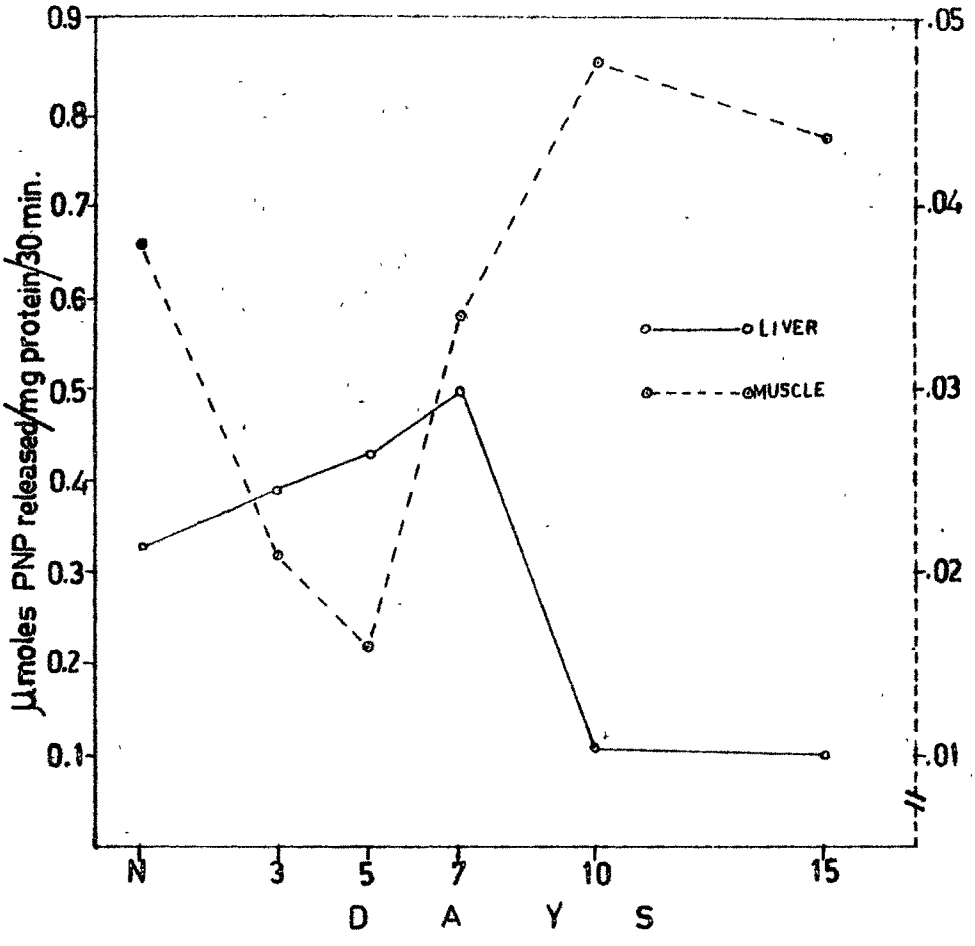

Fig. 6 : Graphic representation of the levels of alkaline phosphatase in the liver and muscle under simultaneous limb amputation and caudal autotomy in H. flaviviridis

level. AA content of the liver fell on 3rd day, and on 10th day it attained the normal level followed by a fall on the 15th day. Tail AA remained at the normal level till 5th day except for a slight increase on the 1st day. Thereafter, the vitamin level gradually increased resulting in an above normal level on 7th, 10th and 15th days.

Acid phosphatase of the muscle and liver showed an increase on the 5th day and a decrease on 10th day followed by a fall on 15th day. In both the enzymes remained at a sub-normal level and showed a similar pattern.

Concentration of alkaline phosphatase of the liver and muscle showed different patterns. Liver alkaline phosphatase level increased till the 7th day followed by a sharp decline on the 10th day, but more or less the same sub-normal level was maintained even on the 15th day. Muscle showed an initial depletion till the 5th day followed by a gradual increase till the 10th day. Though a slight decrease was noted on 15th day, it nevertheless remained in an above normal level.

DISCUSSION

Possible influence of autotomy induced state of the animal on simultaneous amputation of a non-regenerating part

has been looked into in the present investigation. Apparently, the study intended to see whether under the pervading influence of tail regeneration, limb regeneration could be induced; or conversely whether limb amputation would retard tail regeneration, have yielded negative results for both. The various systemic parameters evaluated like tissue protein, glycogen, ascorbic acid; acid and alkaline phosphatase and blood glucose have all showed a greater depletion initially compared to the responses of these parameters noted to occur in the case of tail autotomy or limb amputation alone (Chapter 1-4). Such pronounced depletion indicate the greater physiological stress involved in the healing of the limb wound, and healing and regeneration of the tail, occurring concurrently.

The limb and tail wounds after healing, will lead to epithelialisation and regeneration respectively, and in the period after the first five days, the various parameters depicted an intermediary pattern of modulations with a change more tilted towards that characteristic of tail autotomy and ensuing regeneration. Comparison of the responses of local parameters (Fig. 1-4) indicate the inherent difference in the genetic and physiologic endowments. Obviously, the involvement of local factors in expressing regenerative ability is sufficiently emphasized.

Caudal protein and AA contents showed only slight falls in the initial phase of tail wound healing, and was followed by a higher content with the progression in regeneration. In the limb on the other hand, the above parameters remained sub-normal. Similarly, glycogen depletion in the tail was more pronounced than that in the limb, which was more or less insignificant. Apparently, the similar systemic responses under both, caudal autotomy and limb amputation (Chapters 1-4) and dissimilar local responses as observed presently suggest that an inherent capacity for regeneration lies at the local site.

Though the double stress of simultaneous tail autotomy and limb amputation induced some variations in the degree of systemic responses; the general pattern of changes was more or less identical to those obtained during tail autotomy or limb amputation alone. The fact that under these conditions, the tail could show a normal process of regeneration and the limb no regeneration, gives validity to the earlier conclusion of an inherent potentiality or ability to evoke certain regeneration-specific-factors at the local site. A tentative hypothesis that could be suggested is that, whereas the tail has the necessary local potentiality, the limb does not have; and a successful process of regeneration depends upon both adaptive systemic responses as well as the

necessary local potentiality. Absence of any one of these two factors could lead to loss of regenerative ability. Thus it appears that in an animal which is capable of regeneration of lost part/s, systemic responses are present, while the local potentialities are restricted only to certain regions of the body (capable of regeneration). Again, it may be presumed, that in an animal which has lost the regenerative ability totally, both local potentiality as well as adaptive systemic responses characteristic of the process of regeneration are lost, as in Calotes versicolar (Kinariwala, 1972; Kinariwala et al., 1981; Ramachandran et al., 1978).