CHAPTER 9

IN LOCO AND SYSTEMIC ALTERATIONS IN PROTEIN CONTENT DURING TAIL REGENERATION IN NORMAL, SHAM OPERATED AND GONADECTOMISED LIZARDS, *HEMIDACTYLUS FLAVIVIRIDIS*

During the progress of repair and regeneration, the physiological homeostasis gets altered sufficiently to subserve the synthetic requirements of the biological processes. As the dedifferentiating cells transform into blastemal cells, they are actively engaged in the synthesis of various cellular materials, and energy utilization. The important biochemical process representing growth, is the biosynthesis of proteins and nucleic acids (Rappoport and Fritz, 1972). Nucleic acids are associated with protein synthesis and growth. *In loco* and systemic alterations in nucleic acids and proteins during lizard tail regeneration have been shown in some of the previous investigations from this laboratory (Ramachandran *et al.*, 1980, 1982). In a previous chapter (Chapter 7) gonadectomy was shown to induce faster rate of regeneration. The present chapter attempts to establish the possible correlation between gonadectomy induced better regenerative potential and protein metabolism, if any, by evaluating the temporal quantitative alterations in protein content of the regenerate, liver, muscle, kidney and brain of normal, sham operated and gonadectomised Gekkonid lizards, *Hemidactylus flaviviridis*.

MATERIALS AND METHODS

Adult *H. flaviviridis* collected from the local animal dealer were kept in the laboratory for two weeks to get them acclimated to the new environment. The animals were divided into three groups of equal numbers consisting of both sexes. First group of animals was the intact normal which served as the control. Second group of animals was sham operated (SGX); and third group was gonadectomised (GX) and served as the experimentals. The operative procedures were outlined in Chapter 7. The latter two groups were kept in the cage for 12 days, to minimize the metabolic disturbances, if any, due to surgical stress and ensuing wound healing. At the end of this period, the tails of all the three groups of lizards were autotomized three segments away from the vent. At regular time intervals of 3,5,7,10,15,25,40, and 60 days post-autotomy, the animals were sacrifised, and liver, muscle, tail, kidney and brain protein content was estimated by the method of Lowry *et al.* (1951).

RESULTS

Protein content of the liver in GX, SGX and intact normals showed a more or less similar pattern. The normal ones showed a depletion on 3rd day followed by sudden increase to the normal level on 10th day. Thereafter, there was a gradual depletion in the protein content till the 40th day, followed by an increase on the 60th day. SGX and GX lizards showed a much lower initial protein content in the liver in the pre-autotomic condition. Both showed a rise on the 3rd day followed by a fall on the 5th day. Then onwards the protein level of GX remained higher till the 60th day. SGX animals showed a small peak on 7th day and a fall on 10th day followed by a gradual rise till the 60th day.

Muscle protein content of the controls follow the same pattern as the liver protein except for a lower level on the 25th day and an above normal peak value on the 10th day. SGX animals follow a pattern intermediate between those of normal and GX. The pre-autotomic SGX levels were lower than the controls. Uptill the 10th day, and from thereonwards, it followed a pattern similar to the controls. GX lizards showed a greater increase of muscle protein content in the initial stages. The level gradually increased till the 5th

Table 1. Quantitative alterations in the hepatic protein content (mg/100 mg fresh tissue) during tail regeneration in normal, sham operated and gonadectomised lizards, *H. flaviviridis*.

Periods of regeneration in days	N	3	5	7	10	15	25	40	60	Turnover rate increase/ decrease
Normal intact (IC)	22.662 ±1.89	17.426 ±0.73	17.759 ±2.46	20.032 ±3.93	22.891 ±3.26	21.031 ±3.28	18.447 ±2.06	16.648 ±2.28	20.705 ±0.968	0.76
Sham operated (SGX)	15.631 ±2.0 0.0005@	22.357 ±3.9	13.407 ±2.30	16.525 ±1.13	14.158 ±1.35	15.276 ±2.43	16.821 ±3.46	17.643 ±3.88	18.693 ±2.96	1.80
Gonadectomised (GX)	15.942 ±1.96 0.0025@	20.521 ±2.55	15.325 ±0.304	17.237 ±2.50	17.24 ±2.58	18.55 ±3.46	20.88 ±1.15 0.001*	14.886 ±1.36	15.621 ±3.34	1.29

± S. D.

@ P value in comparison with IC

* P value in comparison with SGX

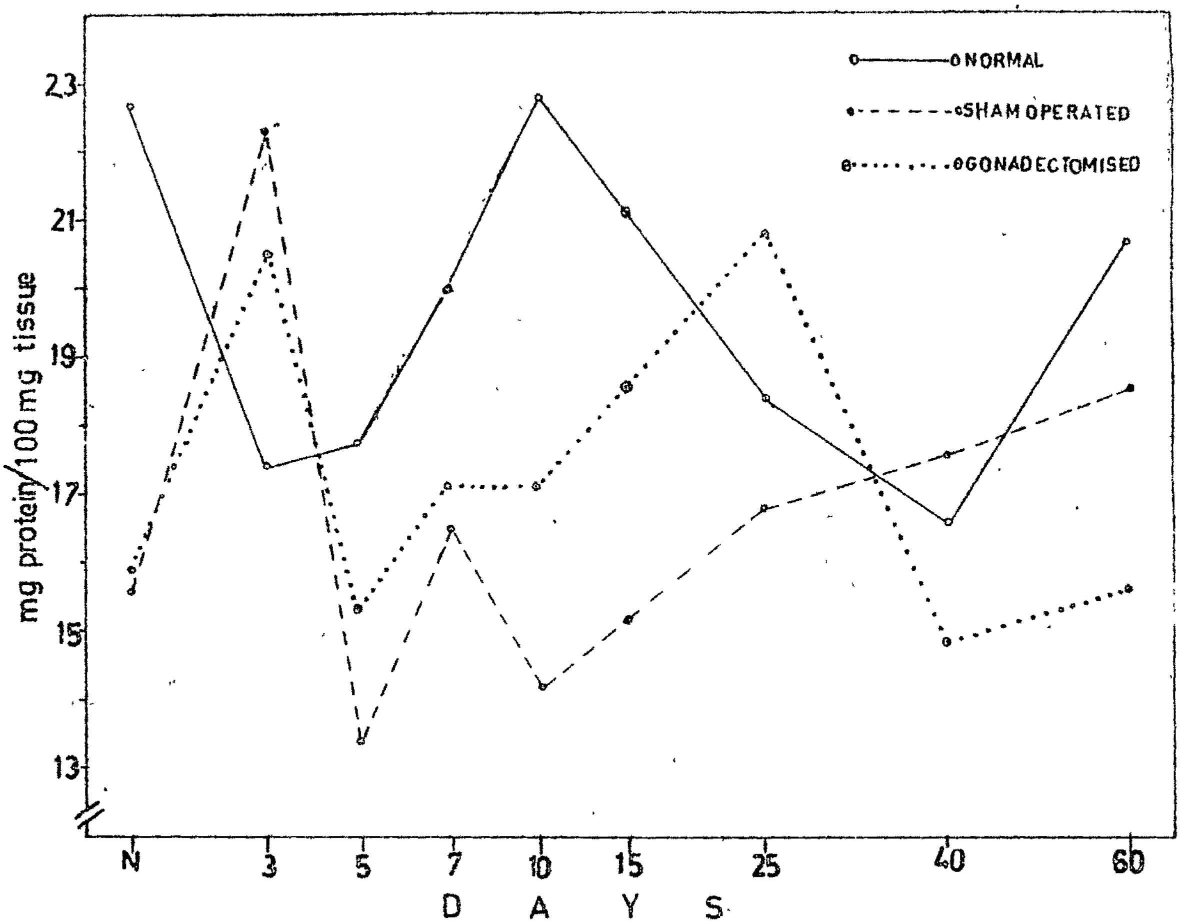

Fig. 1: Graphic representation of the levels of protein in the liver during tail regeneration in the normal, sham operated and gonadectomised lizards, <u>H. flaviviridis</u>

Table 2. Quantitative alterations in the muscle protein content (mg/100 mg fresh tissue) during tain regeneration in normal, sham operated and gonadectomised lizards, <u>H. flaviviridis</u>.

Periods of regeneration in days	N	3	5	7	10	15	25	40	60	Turnover rate increase/decrease
Normal intact (IC)	14.417 ±1.901	12.97 ±0.468	11.302 ±2.67	11.85 ±0.64	17.57 ±4.14	12.17 ±2.63	9.67 ±0.63	13.54 ±0.361	14.22 ±1.62	1.54
Sham operated (SGX)	10.395 ±1.499	9.901 ±1.354	10.189 ±0.451	11.412 ±2.62	7.087 ±0.115	11.109 ±1.527	10.478 ±0.731	14.735 ±1.415	13.699 ±0.445	2.03
Gonadectomised (GX)	7.762 ±2.362 0.005*	11.507 ±1.627	12.307 ±1.671	11.499 ±2.624	8.732 ±2.89	13.545 ±3.89 0.0025*	14.199 ±4.12 0.005*	10.058 ±2.60	9.9 ±2.82	2.04

± S. D.

* P value in comparison with SGX

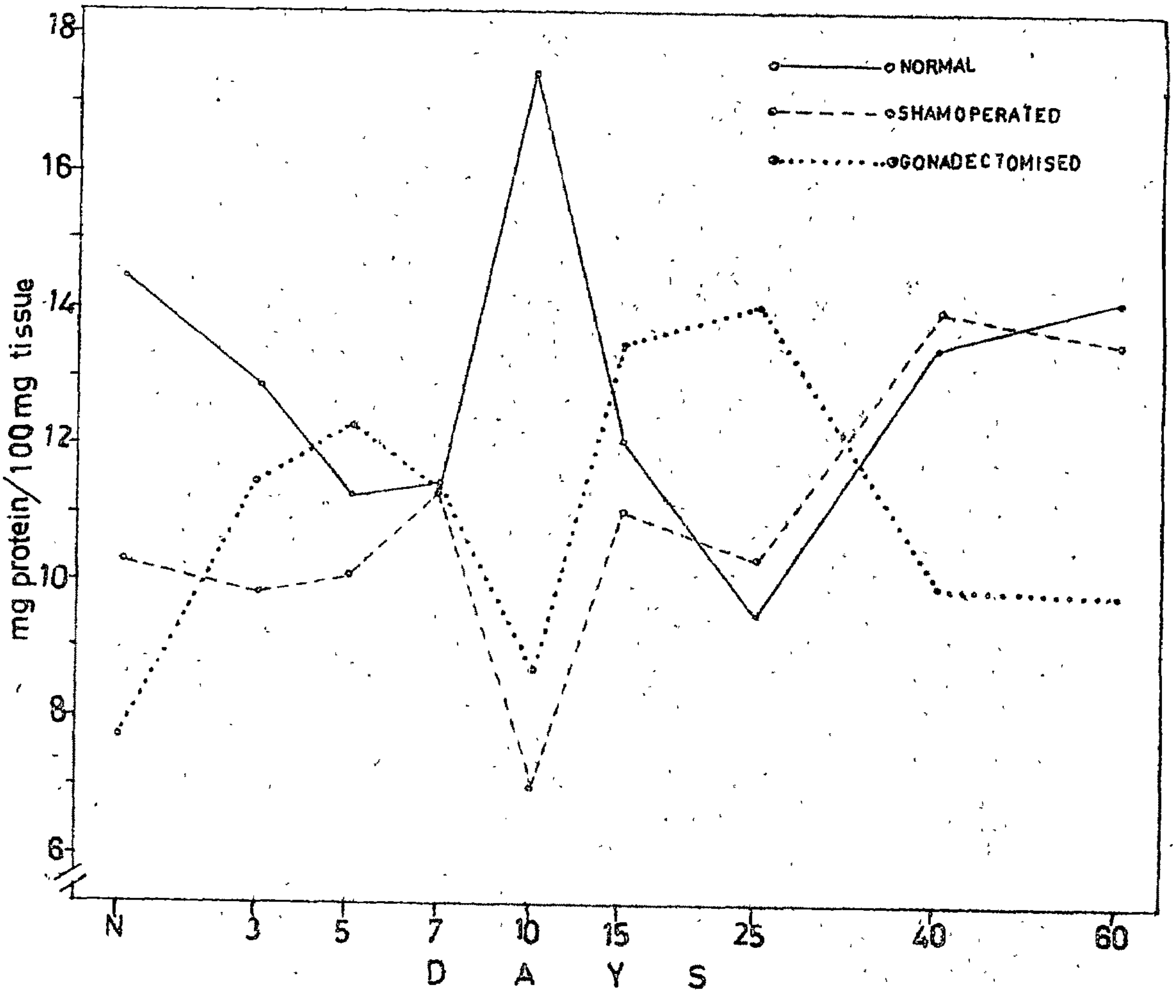

Fig. 2 : Graphic representation of the levels of protein in the muscle during tail regeneration in the normal, sham operated and gonadectomised lizards, H. flaviviridis

Table 3. Quantitative alterations in the caudal protein content (mg/100 mg fresh tissue) during its regeneration in the normal, sham operated and gonadectomised lizards, H. *flaviviridis*.

Periods of regeneration in days	N	3	5	7	10	15	25	40	60	Turnover rate increase/ decrease
Normal intact (IC)	7.014 ±1.034	10.435 ±1.4	8.283 ±0.62	7.36 ±1.79	8.23 ±2.7	7.46 ±0.296	10.026 ±1.63	5.57 ±0.865	8.02 ±2.02	1.66
Sham operated (SGX)	7.39 ±1.58	10.19 ±2.91	5.99 ±0.496	6.34 ±0.95	7.06 ±1.64	8.36 ±1.65	10.076 ±1.91	6.846 ±1.421	7.026 ±1.68	1.85
Gonadectomised (GX)	6.698 ±0.824	6.35 ±0.56	6.7 ±0.61	8.05 ±1.24	7.18 ±0.902	8.32 ±1.49	8.55 ±1.82	9.29 ±0.388	5.92 ±1.74	1.09

± S. D.

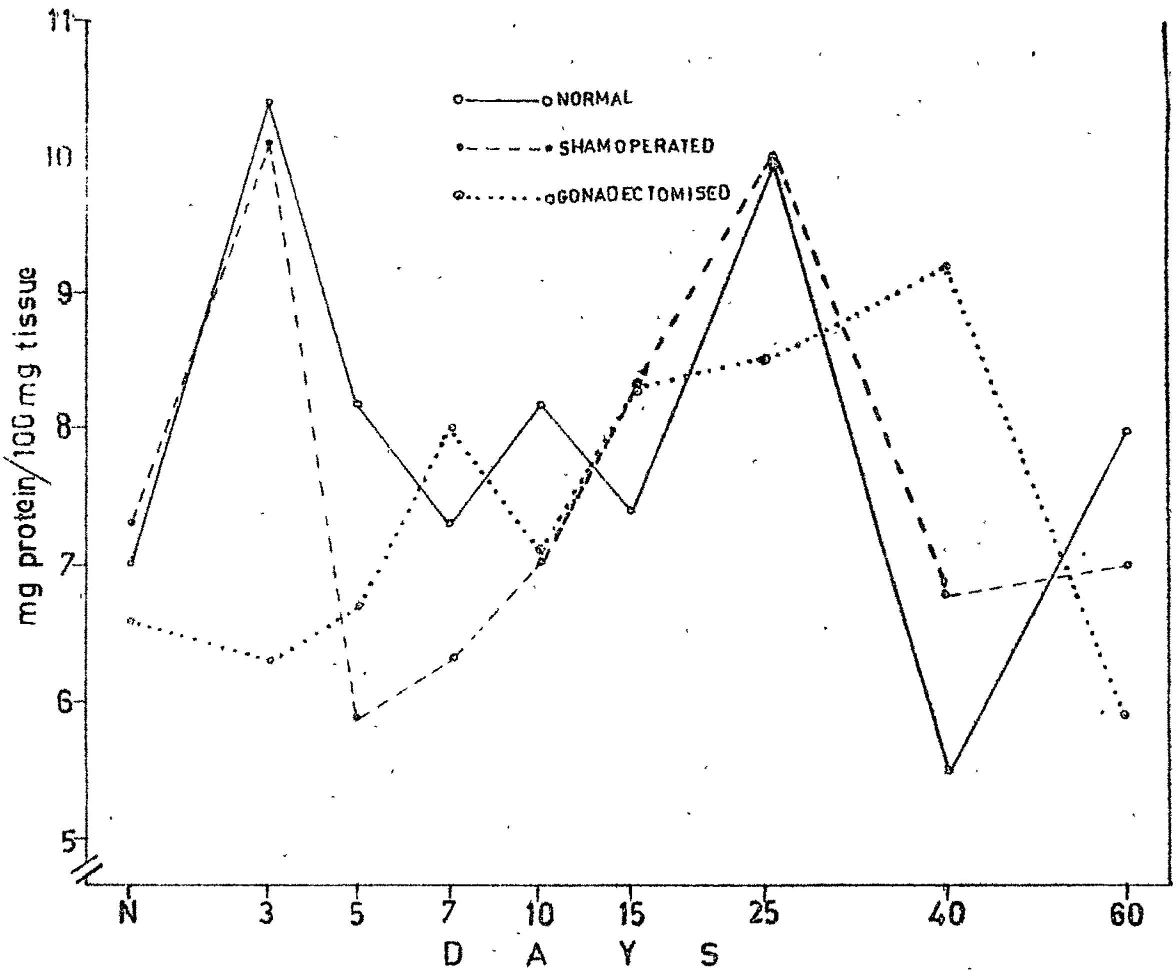

Fig. 3 : Graphic representation of the levels of protein in the regenerating tail during its regeneration in the normal, sham operated and gonadectomised lizards, H. flaviviridis

Table 4. Quantitative alterations in the brain protein content (mg/100 mg fresh tissue) during tail regeneration in the normal, sham operated and gonadectomised lizards, *H. flaviviridis*.

Periods of regeneration in days	N	3	5	7	10	15	25	40	60	Turnover rate increase/ decrease
Normal intact (IC)	12.182 ±1.32	9.58 ±1.01	11.322 ±1.028	10.02 ±1.52	14.59 ±0.53	9.87 ±2.04	7.85 ±0.65	7.43 ±1.82	13.008 ±2.76	1.68
Sham operated (SGX)	8.35 ±0.784	8.51 ±1.12	6.51 ±0.57	8.84 ±1.43	9.34 ±0.77	7.52 ±0.683	7.2 ±0.50	7.52 ±1.24	11.08 ±2.1	1.73
Gonadectomised (GX)	7.61 ±0.89	9.42 ±1.41	7.12 ±0.64	9.09 ±0.875	10.34 ±1.54	11.074 ±1.35 0.005*	11.007 ±1.5 0.005*	8.23 ±1.02	8.64 ±1.47	1.68

± S. D.

* P value in comparison with SGX

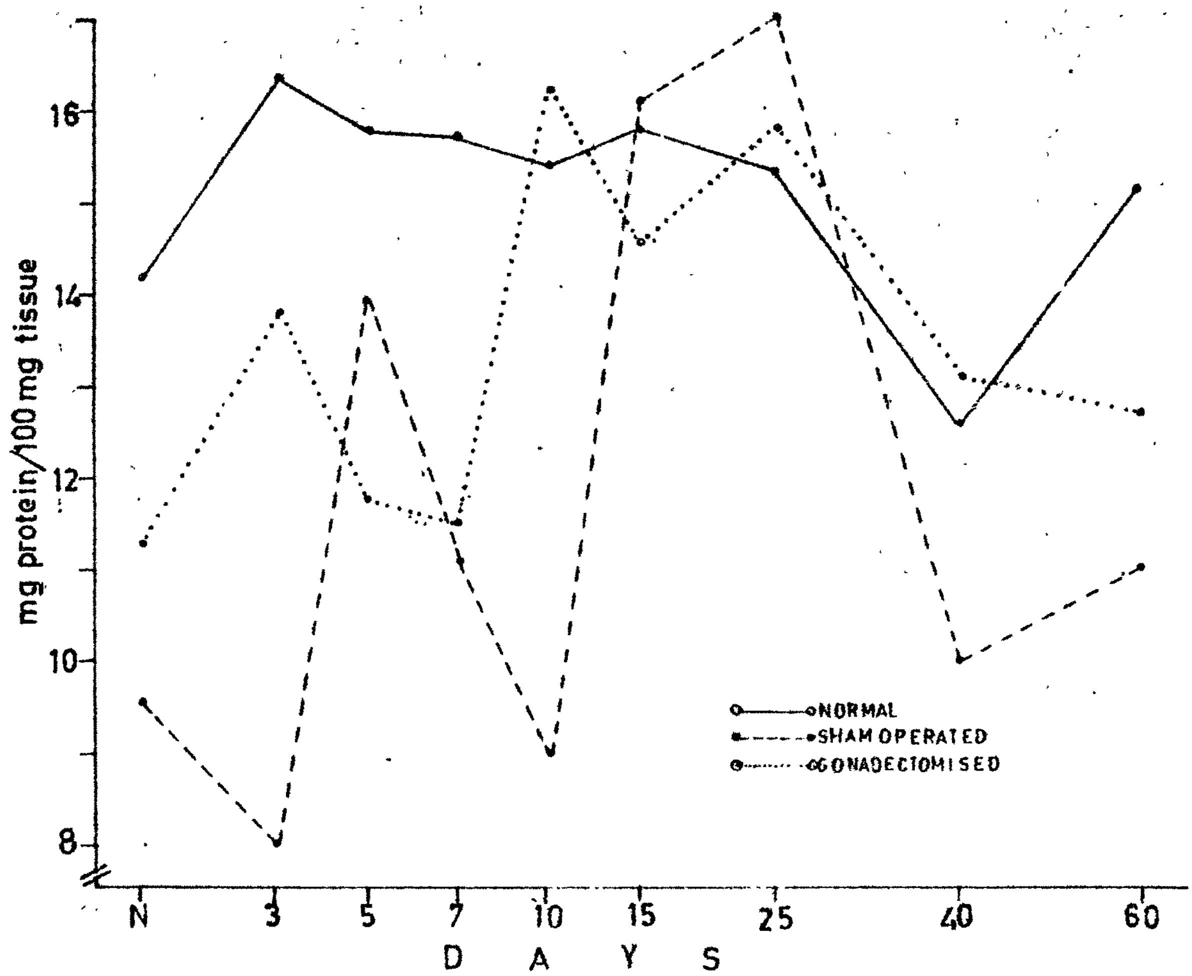

Fig. 4 : Graphic representation of the levels of protein in the kidney during tail regeneration in the normal, sham operated and gonadectomised lizards, H. flaviviridis.

Table 5. Quantitative alterations in the kidney protein content (mg/100 mg fresh tissue) during tail regeneration in the normal, sham operated and gonadectomised lizards, *H. flaviviridis*.

Periods of regeneration in days	N	3	5	7	10	15	25	40	60	Turnover rate increase/ decrease
Normal intact (IC)	14.24 ±3.82	16.42 ±0.404	15.85 ±1.43	15.74 ±1.08	15.48 ±2.24	15.88 ±3.17	15.38 ±1.46	12.86 ±1.70	15.19 ±3.78	1.61
Sham operated (SGX)	9.35 ±1.77	7.99 ±2.04	13.99 ±1.67	11.12 ±0.867	9.04 ±2.45	16.23 ±4.22	17.0 ±4.65	10.47 ±2.95	11.04 ±1.75	2.13
Gonadectomised (GX)	11.30 ±0.97	13.86 ±1.05	11.897 ±2.34	11.50 ±3.41	16.28 ±4.55 0.0005*	14.58 ±2.32	15.896 ±1.68	13.17 ±2.534	12.79 ±1.19	1.44

± S. D.

* P value in comparison with SGX.

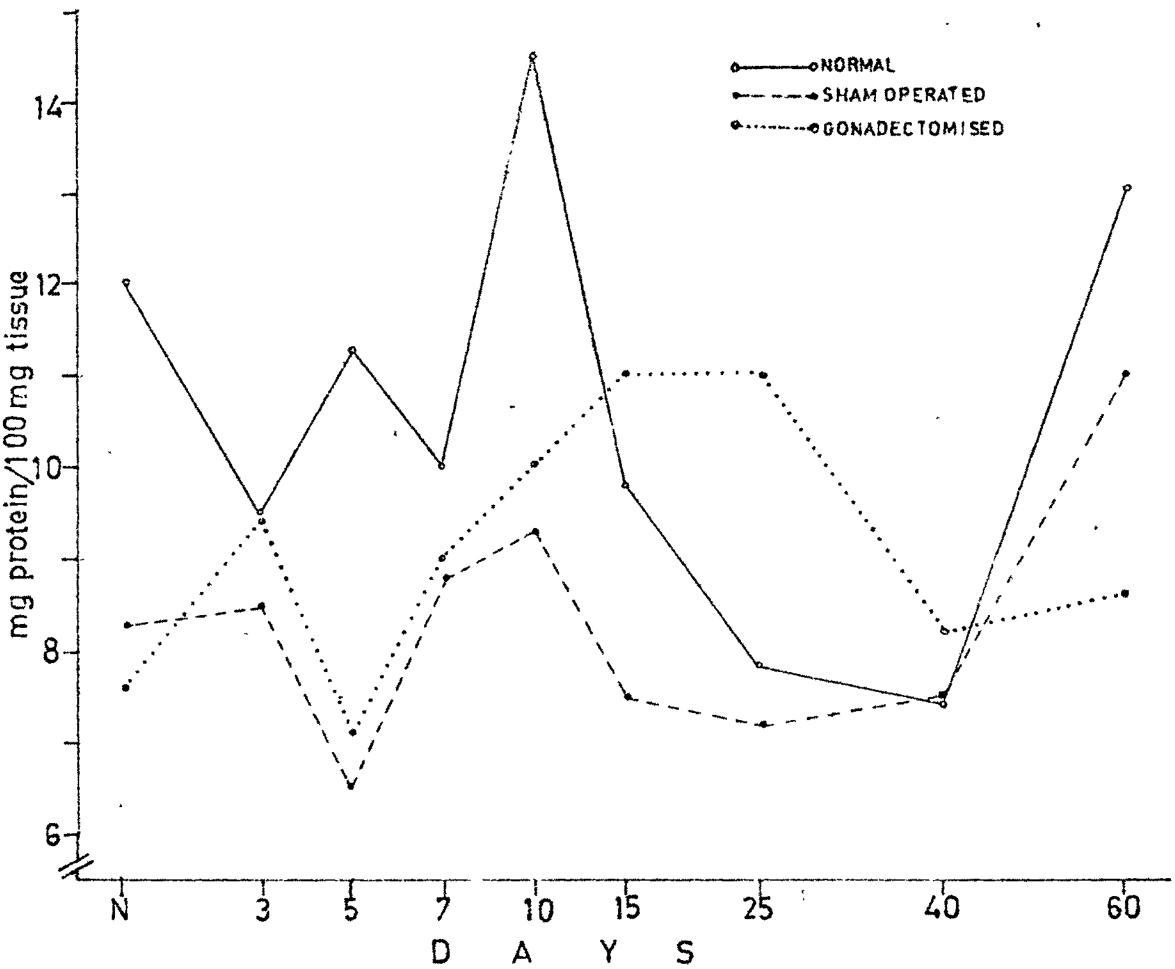

Fig. 5 : Graphic representation of the levels of protein in the brain during tail regeneration in the normal, sham operated and gonadectomised lizards, *H. flaviviridis*

day followed by a fall on the 10th day. The sudden increase on the 15th day continued as a gradual increase till the 25th day which was followed by a fall by the 40th day and was maintained so even on the 60th day.

Tail protein showed a similar pattern in the SGX and control. But the GX lizards showed an initial low level followed by a slight fall (3rd day) and an increase till 7th day and a slight decrease on the 10th day. Thereafter the caudal protein content continued to increase till the 40th day. Between 40th and 60th days there was a significant decrement.

Kidney protein content showed a greater fluctuation in the case of SGX. The intact normals showed minor fluctuations. The protein content of the kidney showed a rise on the 3rd day followed by more or less of continuous decrease till the 40th day and then rose upto the normal level by 60th day. The much low initial level of kidney protein in SGX showed a fall on the 3rd day followed by a sharp rise on the 5th day. It then decreased to the pre-autotomy level by the 10th day and then again decreased to a maximal level by the 25th day. Between 25th and 40th days there was a conspicuous drop and then increased slightly by the 60th day. The intermediate initial level noticed in the case of GX lizards showed continuous rise and fall resulting in three peak values on 3rd, 10th and 25th days. From 25th day onwards there was a continuous decline, till the level on the 60th day was very much close to the pre-autotomy value.

Brain protein content of the control lizards showed a depletion on 3rd day post-autotomy, a slight rise on 5th day followed by a fall again on the 7th day. By 10th day the protein content increased to a peak level which was followed by a gradual fall till the 40th day resulting in the attainment of a minimum value. By the 60th day, the protein content had increased towards the normal level. The SGX and GX lizards showed a more or less similar pattern with an initial set of changes during the first week being exactly opposite to that of the controls. From the 7th day onwards, the pattern of changes in both SGX and GX were more or less identical to the controls except for different levels of protein in the two cases. The changes outlined above are represented in figures 1-5 and tables 1-5.

DISCUSSION

Definite alterations in systemic protein turnover in relation to caudal autotomy and ensuing regeneration can be deduced from the present results. Similar observations were made with respect to tail regeneration in *M. carinata* also

(Ramachandran et al., 1980, 1982). However, there appears to be a different mode of protein turnover in H. flaviviridis as compared to M. carinata. A net positive nitrogen balance with increased protein content in the regenerate, as well as liver and muscle was the feature in M. carinata (Ramachandran et al., 1980). The present results tend to indicate a net negative nitrogen balance in the body as a whole as marked by below normal levels of protein almost throughout the regenerative period in the various organs studied. Expectedly, the tail regenerate depicted increasing protein content.

SGX and GX animals in general tended to have decreased protein content in brain, kidney and muscle indicating a generalised protein depletion following surgical stress. Brain and muscle appear to be more sensitive to gonadectomy as noted by the further decreased protein content in GX lizards as compared to the SGX ones. Gonadectomy tended to have a nullifying effect on surgical stress induced protein depletion in kidney. Except for liver and tail, all the other organs had a reduced protein content in SGX and GX lizards in the pre-autotomy condition.

Subsequent to tail autotomy, all the three groups of lizards showed more or less identical pattern of modulations in protein content except for temporal shifts in the various spikes. Keeping the corresponding pre-autotomy levels as the basal levels, both controls and SGX lizards maintained more or less below basal levels of protein. However, in GX lizards, most of the modulations remained above the basal level. Regeneration induced modulations in the systemic and _in loco_ protein contents seem to be affected by the absence of gonadal steroids. In fact the better growth rate observed in GX lizards (Chapter 7) could be due to the balanced protein turnover in the body. Protein turnover value expressed as the ratio of percentage increase to percentage decrease for the whole period of regeneration was maximal in SGX lizards. This could have some correlation with the observed better regenerative growth in comparison to the controls. It is significant that the turnover value for the liver was below unity in controls which may be responsible for the slowest growth rate observed. Moreover, the higher values obtained for muscle in SGX and GX lizards may again be of some significance in this respect. Another pertinent aspect is the almost arrested regenerative growth in GX lizards between the 25th and 40th days post-autotomy (Chapter 7). The present results on protein contents have shown an opposite pattern of alterations in GX lizards in comparison to SGX and control ones during this period. The protein content of the regenerates fell drastically, and that of muscle increased in control and SGX lizards, while in the GX lizards, the muscle protein content decreased, and the tail protein content showed gradual increase (Fig.3). Could the fall in protein content in the regenerate in the controls have some critical influence on promoting late differentiation and growth events, and the delay in such a fall be responsible for the observed plateauing out of growth rate in gonadectomised lizards? This is a query which cannot be answered at present. However, it is safe to presume from the present study that the gonads and their steroid output are non-essential for bringing about systemic and *in loco* modulations in protein content associated with regeneration. Further, it may also be speculated that in the absence of gonadal steroids the sensitivity and response to thyroid and adrenal hormones are potentiated which could be a significant factor in the better regenerative ability exhibited by the GX lizards.