

CHAPTER 8

IN LOCO AND SYSTEMIC ALTERATIONS IN GLYCOGEN CONTENT AND BLOOD GLUCOSE LEVEL DURING TAIL REGENERATION IN RELATION TO GONADECTOMY IN GEKKONID LIZARD, HEMIDACTYLUS FLAVIVIRIDIS

Fundamentals of the biodynamics of the regenerating systems, such as the understanding of the molecular events and controlling mechanisms that direct the regeneration of tissues, organs and complex organised structures, such as tail or limb have long been a challenge. Recent studies on tail regeneration in lizards from this laboratory have emphasized the contribution of the systemic factors (metabolic and other) as supplementary support for the regenerative mechanisms in operation at the local site. Endocrine participation comes as a natural correlate to these alterations; hence investigations on those lines also have been initiated. Significant involvement of thyroid in lizard tail regeneration has as a result been envisaged (Swami, 1982). In another similar study the atrophy of gonads and retardation of regeneration subsequent to surgical thyroidectomy in H. flaviviridis was construed to indicate in the possible involvement of gonadal steroids in regeneration (Shah et al., 1979). Moreover, the gonadal steroids (androgens and estrogens) are known to have

anabolic effects involving carbohydrates (Chandola et al., 1974; Spellacy, 1979; Wynn and Doar, 1970; Felig, 1977; Shamoon and Felig, 1974; Sanchez, 1977; Deshpande et al., 1978; Gangaramani, 1979). The present study attempts to link the above two sets of alterations if any, in the carbohydrate stores of the body like liver, muscle and tail glycogen, and blood glucose in gonadectomized lizard Hemidactylus flaviviridis in relation to tail regeneration.

MATERIALS AND METHODS

After allowing a fortnight of acclimat~~ion~~ion to laboratory conditions, the lizards Hemidactylus flaviviridis (collected from the local animal dealer) in the weight group 10-12 grams were divided into three groups. One group of animals was left as such to serve as intact controls, while the second group consisted of those which were sham operated (SGX) and the third group was those which were gonadectomized (GX). Gonadectomy and sham operation were performed as outlined in Chapter 7. After a time lapse of 12 days (for healing of the surgical wound) all the three sets of animals were subjected to caudal autotomy and kept in different cages. At regular intervals of 3,5,7,10,15,25, 40 and 60 days post autotomy, 6 lizards from each group were sacrificed and liver, muscle and regenerating tail

were removed, weighed and used for glycogen estimation by the method of Seifter et al. (1955). Blood was collected by cardiac puncture and glucose level was estimated by the micromethod of Folin and Malmros (1929).

RESULTS

The pre-autotomy level of blood glucose was low in both GX and SGX groups with a much lower level in SGX lizards. Subsequent to tail autotomy, all the three groups of animals showed a fall in blood glucose level with normal intact controls showing a continuous fall lasting upto 15th day, whence the lowest level was reached. In contrast, the GX and SGX lizards showed the attainment of lowest levels of blood glucose by 3rd and 5th days respectively. Subsequently, an elevation in blood glucose was noticeable in both these groups with pre-autotomy levels being attained by 7th and 10th days respectively. Thereafter, both the groups again showed a fall in blood glucose ultimately reaching levels characteristic of the normal controls by 15th day. From 15th day onwards all the three groups depicted a continuous and gradual increase in blood glucose level till the 60th day.

Alterations in the systemic glycogen content show an active participation of both hepatic and caudal stores of

Table 1. Alterations in blood glucose level (mg/100 ml) 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
Normal intact (IC)	118.919 ± 8.056	116.216 ± 11.466	86.48 ± 10.81 < 0.0005 [@]	84.486 ± 5.875 < 0.0005 [@]	84.33 ± 7.17	68.315 ± 13.01 < 0.0005 [@]	85.135 ± 5.733	75.648 ± 13.865 < 0.0005 [@]	108.96 ± 10.586
Sham operated (SGX)	81.08 ± 10.01 < 0.0005 [@]	62.162 ± 13.48 < 0.0005 [@]	70.216 ± 3.82	86.515 ± 3.821	86.488 ± 10.809	108.108 ± 15.289	83.00 ± 2.82	80.00 ± 8.108	106.801 ± 2.777
Gonadectomised (GX)	91.891 ± 14.30 < 0.0005 [@] < 0.0005 [*]	80.985 ± 9.787	62.162 ± 8.289 < 0.0005 [@]	70.267 ± 5.435 < 0.0005 [@]	89.86 ± 11.104	68.477 ± 9.478 < 0.0005 [*]	76.675 ± 11.781 0.005 [*]	84.682 ± 15.018	102.667 ± 16.67

± S. D.

@ P value obtained in comparison with IC

* P value obtained in comparison with SGX

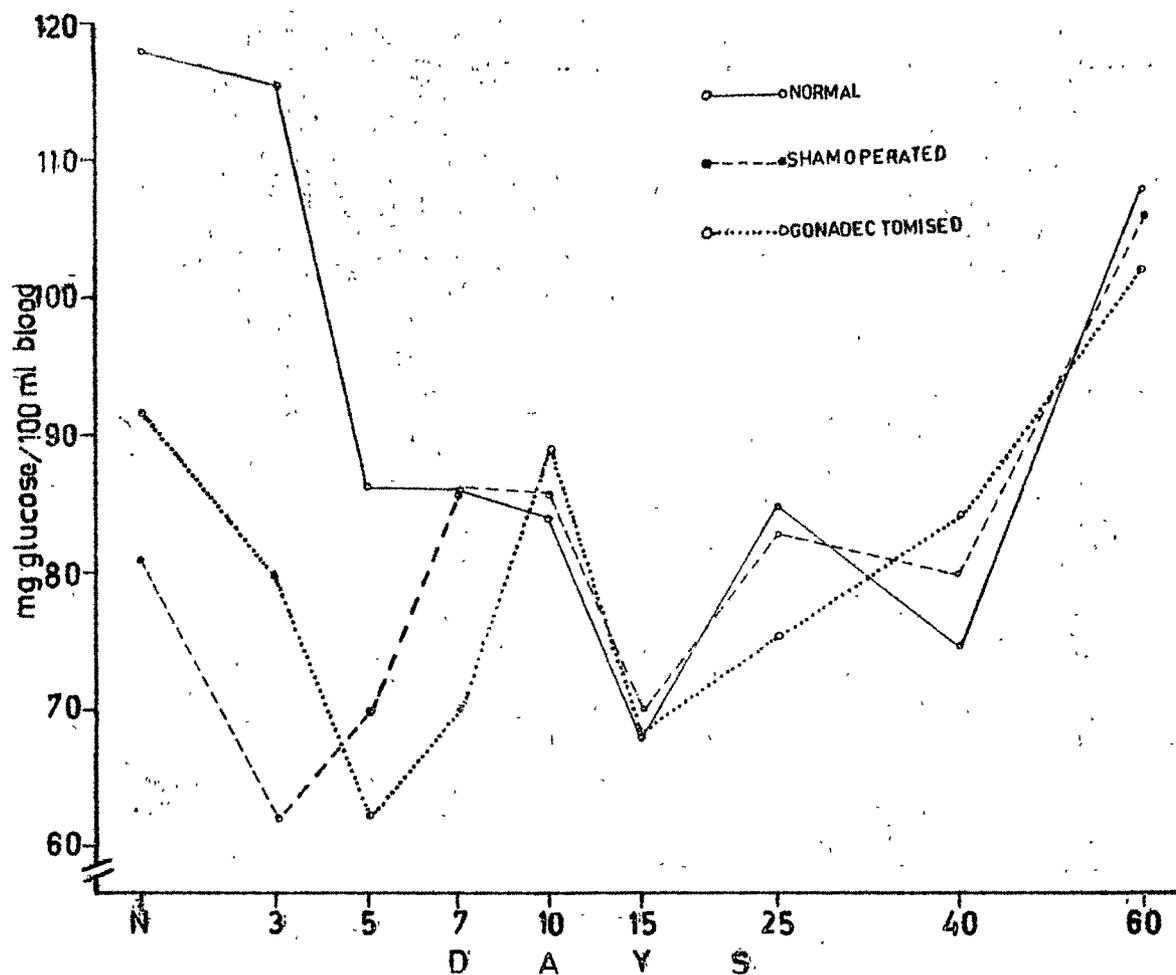


Fig. 1 : Graphic representation of the levels of blood glucose during tail regeneration in the normal, sham operated and gonadectomised lizards, H. flaviviridis

Table 2. Changes in the hepatic glycogen (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
Normal intact (IC)	1.4285 ±0.851	0.5145 ±0.267 0.0005@	0.7057 ±0.471 0.0025@	1.8284 ±0.414	1.560 ±0.308	1.697 ±0.458	0.6758 ±0.198 0.001@	0.399 ±0.0146 0.0005@	0.7025 ±0.371
Sham operated (SGX)	0.121 ±0.0262 0.001@	0.555 ±0.0329	0.871 ±0.0243	0.5044 ±0.1276	0.579 ±0.182	0.662 ±0.0796 0.0005@	0.5187 ±0.0468 0.002@	0.677 ±0.1719 0.0005@	0.75 ±0.067
Gonadectomised (GX)	0.679 ±0.204 0.0025*	0.821 ±0.182	0.4039 ±0.167	1.270 ±0.282	0.5086 ±0.228	1.210 ±0.063	0.433 ±0.137 0.0025*	1.614 ±0.328	0.689 ±0.187

± S. D.

@ P values obtained in comparison with IC

* P values obtained in comparison with SGX

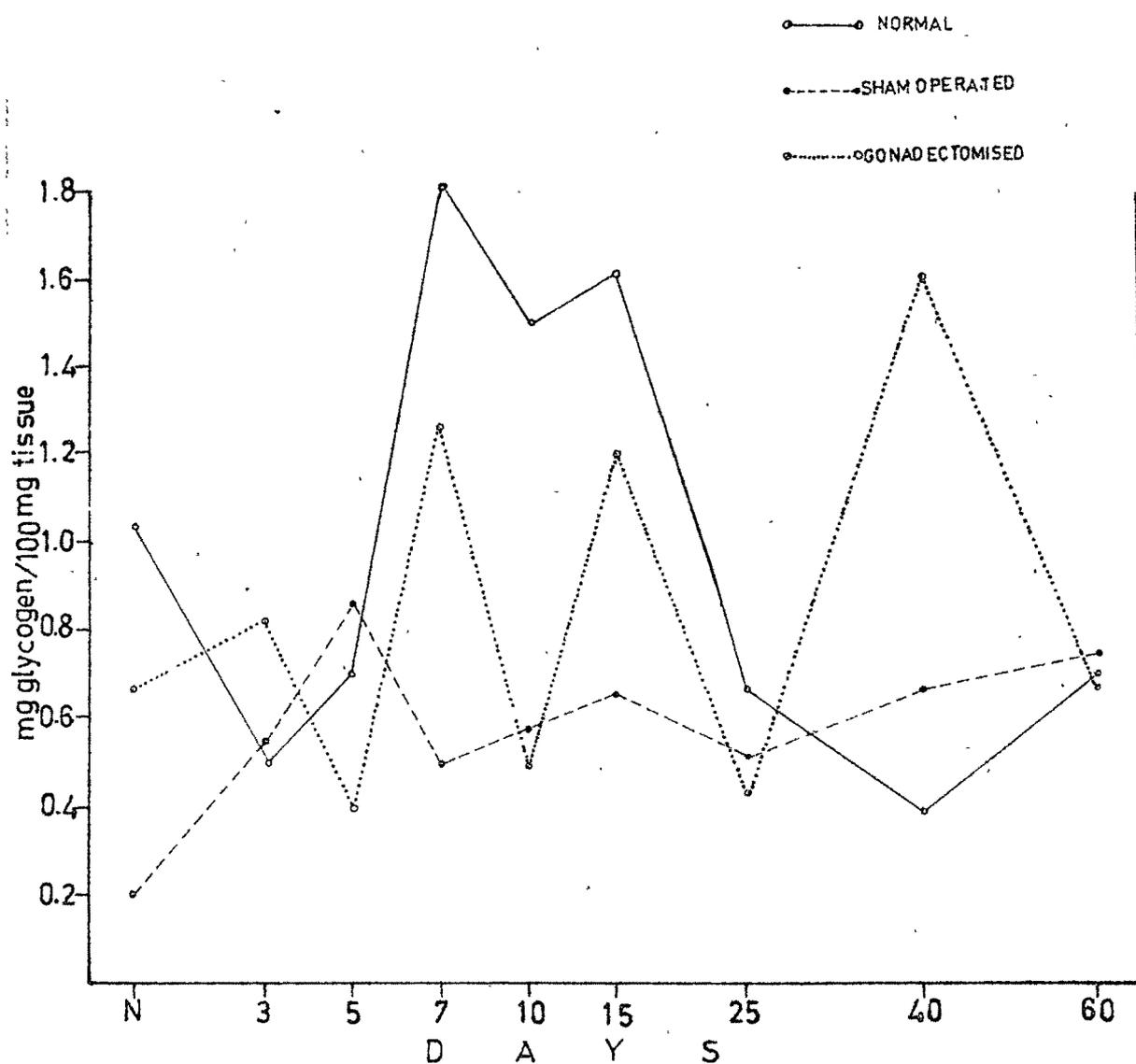


Fig. 2 : Graphic representation of the levels of glycogen in the liver during tail regeneration in the normal, sham operated and gonadectomised lizards, H. flaviviridis.

Table 3. Changes in the muscle glycogen 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
Normal intact (IC)	0.08187	0.1184	0.127	0.188	0.3398	0.142	0.312	0.114	0.296
	± 0.02014	± 0.0142	± 0.0463	± 0.0743	± 0.0294	± 0.0229	± 0.028	± 0.0815	± 0.119
Sham operated (SGX)	0.1889	0.154	0.125	0.364	0.210	0.1845	0.2516	0.2165	0.2147
	± 0.040	± 0.059	± 0.037	± 0.049	± 0.035	± 0.0105	± 0.0382	± 0.0186	± 0.0152
Gonadectomised (GX)	0.001 [@]			0.001 [@]					
	0.0025 [@]								
Gonadectomised (GX)	0.229	0.125	0.326	0.1717	0.2611	0.237	0.170	0.1431	0.158
	± 0.0213	± 0.04	± 0.06	± 0.096	± 0.10	± 0.08	± 0.017	± 0.047	± 0.046
	0.0025 [@]		0.001 [*]		0.0025 [*]				
	0.0025 [*]								

\pm S. D.

[@] P values obtained in comparison with IC.

^{*} P values obtained in comparison with SGX.

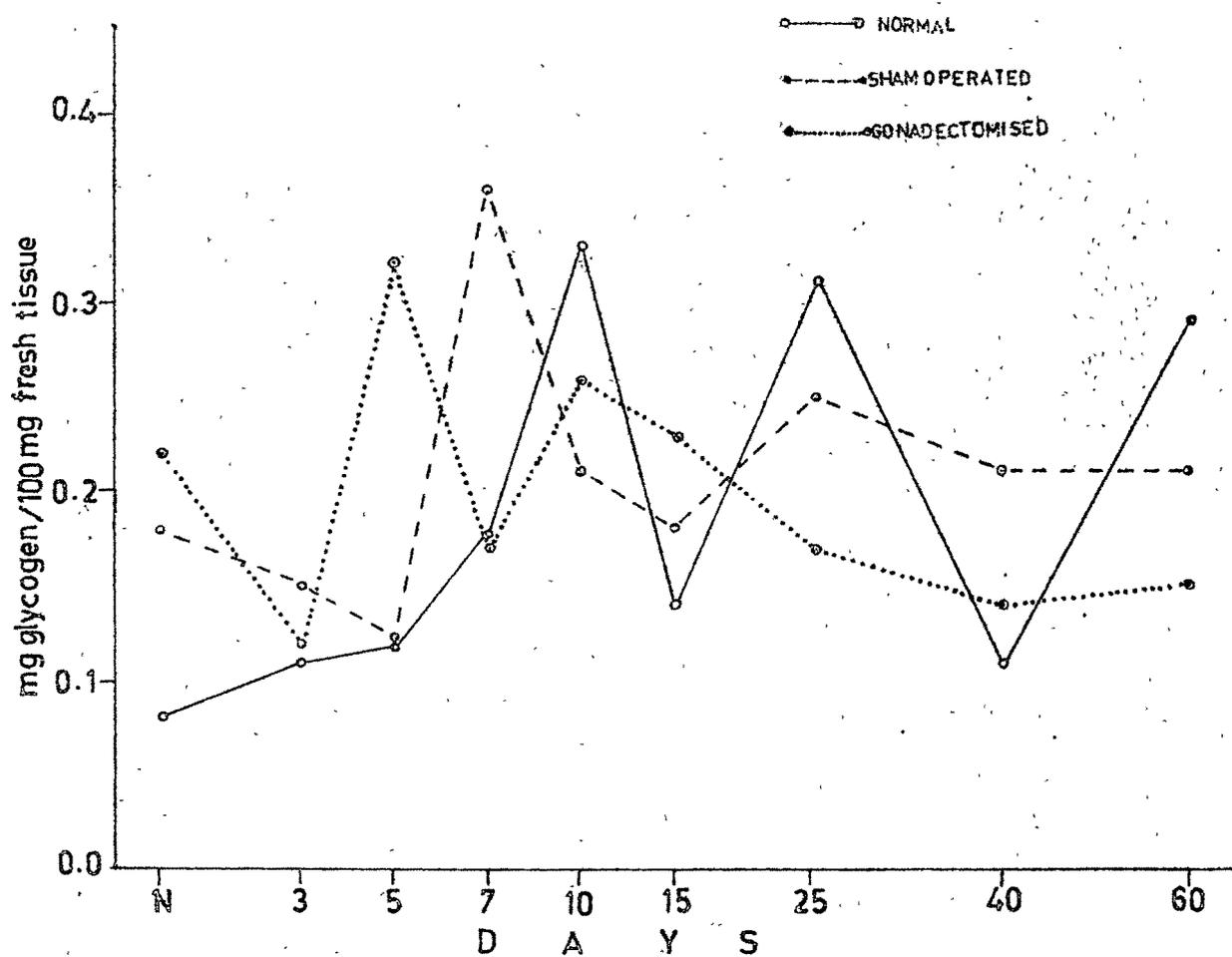


Fig. 3 : Graphic representation of the levels of glycogen in the muscle during tail regeneration in the normal, sham operated and gonadectomised lizards, H. flaviviridis.

Table 4. Changes in the caudal glycogen content (mg/100 mg fresh tissue) during its regeneration in normal, sham operated and gonadectomised lizards, H. flaviviridis.

Periods of regeneration in days	N	3	5	7	10	15	25	40	60
Normal intact (IC)	0.3903	0.143	0.169	0.213	0.825	0.119	0.433	0.333	0.381
	± 0.025	± 0.03	± 0.10	± 0.07	± 0.039	± 0.06	± 0.013	± 0.04	± 0.07
Sham operated (SGX)	0.0845	0.0894	0.178	0.706	0.1459	0.575	0.372	0.407	0.298
	± 0.006	± 0.028	± 0.08	± 0.12	± 0.03	± 0.02	± 0.022	± 0.127	± 0.03
Gonadectomised (GX)	0.182	0.259	0.142	0.356	0.206	0.195	0.309	0.105	0.615
	± 0.006	± 0.005	± 0.05	± 0.03	± 0.015	± 0.012	± 0.024	± 0.023	± 0.12
	0.0025 [@]					0.0005 [*]	0.0005 [*]		
	0.001					0.0005 [@]	0.0005 [@]		

\pm S. D.

[@] P values obtained in comparison with IC

^{*} P values obtained in comparison with SGX

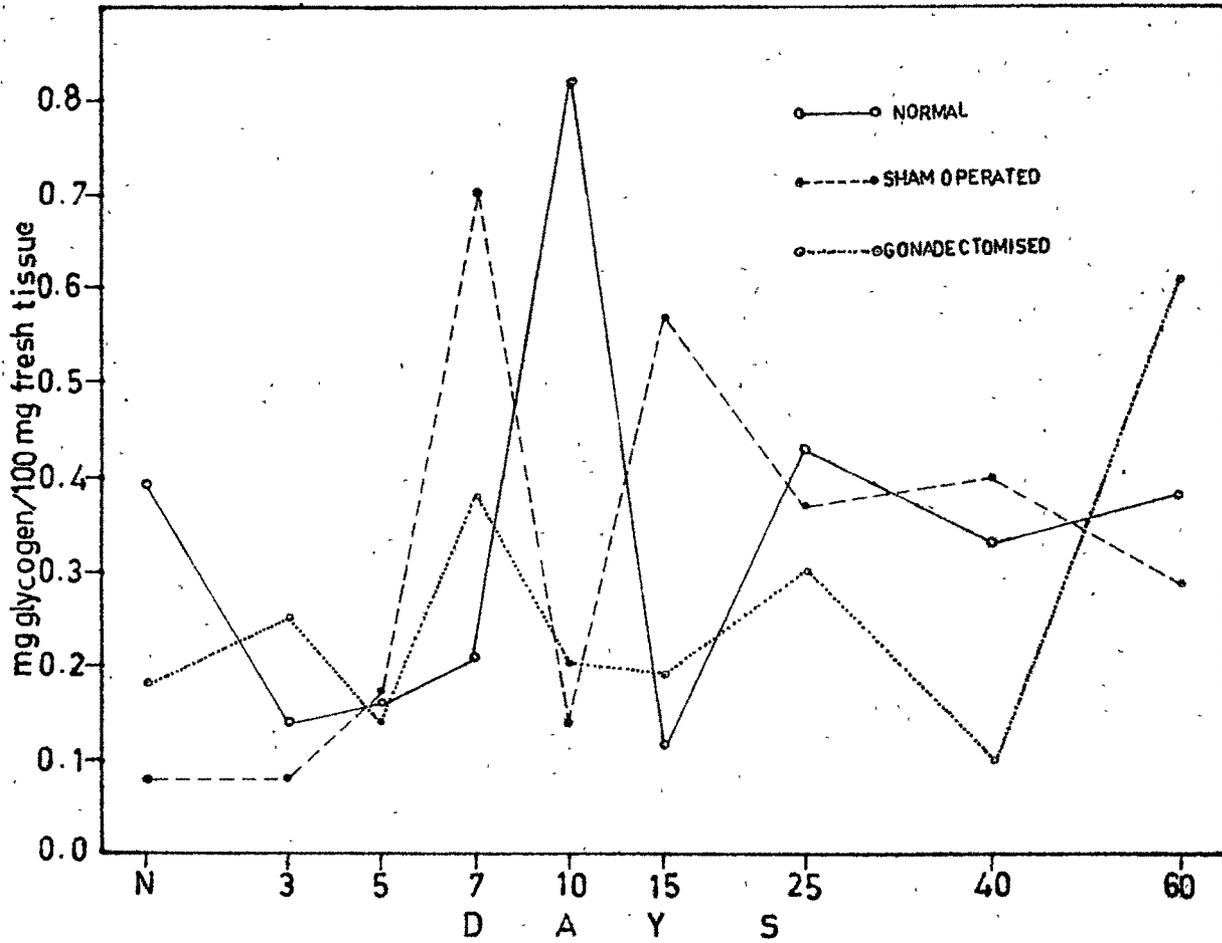


Fig. 4 : Graphic representation of the levels of glycogen in the regenerate during tail regeneration in the normal, sham operated and gonadectomised lizards, H. flaviviridis.

this metabolite during normal tail regeneration, while in the gonadectomized animals moderate utilization from liver, muscle as well as tail stump appear to be the feature. Basal levels of the metabolite in pre-autotomy phase appeared to be reduced in liver and tail of GX and SGX groups of lizards while in the muscle both the groups depicted elevated levels. The phase (regeneration) specific alterations in tissue glycogen and blood glucose in all the three groups are depicted in tables 1-4 and figures 1-4.

DISCUSSION

Previous studies on tail regeneration have shown increased anaerobiosis in loco and glycogen depletion from the visceral organs in the initial periods of post-autotomy (Shah et al., 1977) followed by a phase of glyco/gluconeogenesis in the second week and a further depletion during the 3rd and 4th week (Shah et al., 1977). Similar pattern of changes are shown by the liver and tail glycogen contents of the controls in the present study too. However, the pattern of changes exhibited by muscle glycogen is quite in contrast with that observed in Mabuya carinata. In the present study on H. flaviviridis, muscle glycogen was never below normal and was marked by increasing content all throughout except for two falls in GX and SGX between 10th

and 15th, and 25th and 40th days respectively. Significant depletion in muscle glycogen was the feature in M. carinata in the first half of the regeneration period, which was correlated with aminoacid biogenesis (Ramachandran et al., 1982). H. flaviviridis which has perfected a different metabolic adaptation appears to favour utilization of blood glucose as noted by the continuous fall in blood glucose till 15th day post-autotomy. This is in contrast to that in M. carinata where significant hyperglycemia was the feature (Shah et al., 1982). Glycogen oriented carbohydrate metabolism in M. carinata (Shah et al., 1977) as opposed to insulin dependent metabolism in H. flaviviridis (Miller and Lagios, 1970) seem to be responsible for the observed difference in the metabolic modus operandi between the two lizards.

Both SGX and GX lizards showed a reduced blood glucose, and liver and tail glycogen contents in the pre-autotomy condition. However, the levels in SGX were lower than in GX, thus indicating the nullifying influence of GX condition on sham operation induced hypoglycemia and glycogen depletion. But muscle glycogen was elevated in both GX and SGX with GX having a more pronounced effect. Immediate post-autotomy period was marked by depleting hepatic and caudal glycogen stores of controls while in SGX and GX lizards it was the

muscle glycogen which was depleted. Hypoglycemia was the characteristic feature in all the three groups. But in the SGX and GX groups, unlike in the control, there was swift elevation of blood glucose between 3rd and 7th, and 5th and 10th days respectively which could be due to the blood glucose homeostatic mechanism being activated because of the attainment of the lowest threshold levels. The general pattern of changes from the 7th day onward appears to be more or less similar in all the three groups. However, the levels of tissue glycogen had a tendency to be low in the GX lizards all throughout except between 25th and 40th days whence hepatic glycogen was highly elevated (instead of depletion) and between 40th and 60th days whence the glycogen content in the tail regenerate was tremendously increased. Though it is difficult to explain these changes, the hepatic glycogenetic phase was incidentally marked by very much reduced regenerative growth (Chapter 7).

The faster rate of growth obtained for SGX lizards (Chapter 7) cannot be explained easily from the present observations on alterations in tissue glycogen and blood glucose levels, though it may be mentioned that the modulations and spikes in the glycogen contents were seen to occur earlier in SGX lizards as compared to the controls. It is also worth noting, that hepatic glycogen content was found to be lowest in SGX lizards all throughout. From the

present observations it may be summarised that the surgical stress (Gonadectomy and Sham operation) induced alterations in lizards are more prolonged and that an animal with the regenerative potential evoke the necessary systemic support even at times of injury to a body part. This should facilitate the speeding up of the regenerative potential when there is subsequent loss of the part capable of undergoing regeneration. In the absence of gonadal steroids such a state might prove to be still more permissive and conducive to the regenerative growth (Chapter 7).