CHAPTER 11

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

Interest in the role of lipids in the regenerative mechanics of reptiles started with the work of Woodland (1920) who observed large quantities of lipid in the submuscular and subcutaneous (lateral side only) adipose tissues of the tail of the lizard *H. flaviviridis*. Later Chakko (1968), Radhakrishnan (1972), Shah and Hiradhar (1977), and Chakko and Mariamma (1981) demonstrated quantitative and histochemical alterations in lipids during tail regeneration in *Mabuya carinata* and *Hemidactylus flaviviridis*. Gonadectomy has been shown to induce fat deposition in the subcutaneous muscle layer and in the liver of rat (Tarttelin *et al*., 1975; Gangaramani, 1979). Nathaniel and Nathaniel (1966) observed an increase in lipid content of the cells during a study of cytological changes in the liver after gonadectomy of male rabbits. According to Watkin *et al*. (1972) hepatic output of triglycerides is regulated by gonadal or gonadotropic hormones. Estradiol treatment of both males and females and testosterone treatment of male rats for 3 days have been reported to lower fatty acid synthesis and increase lipolysis. Ovariectomized rats showed loss of metabolic oscillations while fatty acid synthesis remained higher (Molgaard *et al.*, 1980). Concentration of plasma cholesterol and triglycerides have increased after ovariectomy (Van Lenten *et al.*, 1981). Ethynyl oestradiol markedly decreases lipase activity in the liver (Valette *et al.*, 1977). These observations along with the reports of estrogen receptors in the liver (Chamness *et al.*, 1975; Beers and Rosener, 1977; Norstedt *et al.*, 1981) suggest that estrogens exert considerable influence over hepatic metabolism of lipids. The above citations reveal the involvement of gonadal hormones in lipid metabolism in the body as a whole, specifically in liver. Since the earlier studies from this laboratory have demonstrated not only alterations in *in loco* lipid metabolism but also in the liver as part of a systemic response during tail regeneration in lizards (Kinariwala *et al.*, 1978; Ramachandran *et al.*, 1979; Shah *et al.*, 1982), effect of gonadectomy on lipid metabolism in relation to tail regeneration has been considered necessary to study. Hence, alteration in the content of total lipids, phospholipids, cholesterol and triglycerides have been quantitatively assayed in the regenerate and liver during various stages of tail regeneration in the Gekkonid lizard, *Hemidactylus flaviviridis*.

MATERIALS AND METHODS

Adult H. flaviviridis of 10-12 grams weight, were collected from the local animal dealer. They were kept in suitable cages under laboratory conditions on a diet of insects. After a fortnight of acclimation, they were divided into three groups (C, SGX, GX) as outlined in the previous chapters and the tails autotomised twelve days post-surgery. The surgical procedure was as described in the previous chapter (Chapter 7). The lizards were then sacrifised at regular time intervals as specified in the present study corresponding to the various phases of tail regeneration. Liver and tail tissues were weighed immediately and kept for lipid extraction in 2:1 chloroform:methanol mixture as per the method of Folch et al. (1957). Phospholipids and cholesterol were estimated as per the methods of Dittmer and Wells (1969) and Crawford (1958) respectively.

RESULTS

Total lipids : The lipid content of the liver in all the three groups of lizards showed a similar pattern, with the GX showing an elevated level, normal ones an intermediate level, and the sham operated the minimum level. During the course of regeneration, hepatic lipid content was high during the first five days in all the three groups, which

Table 1. Changes in total hepatic content (mg/gm 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)	194.5 ±3.42	242.0 ±4.28	320.9 ± 7.2	155.6 ±2.11	185.3 ±5.04	116.5 ±3.05	134.0 ±1.48	136.9 ±4.27	145.0 ±1.824
Sham operated (SGX)	173.3 ±4.71	231.5 ±7.47	276.7 ±8.92	132.8 ±3.52	173.8 ±4.23	120.0 ±4.87	142.2 ±2.78	155.0 ±3.91	130.0 ±4.43
Gonadectomised (GX)	249.5 ±4.44 $<0.0005^*$	333.0 ±8.54	479.6 ±5.66	310.0 ±7.09	298.0 ±6.8	316.7 ±8.5 $<0.0005^*$	222.3 ±5.62 $<0.0005^*$	261.4 ±2.28	208.08 ±3.86

± S. D.

* P value obtained in comparison with SGX

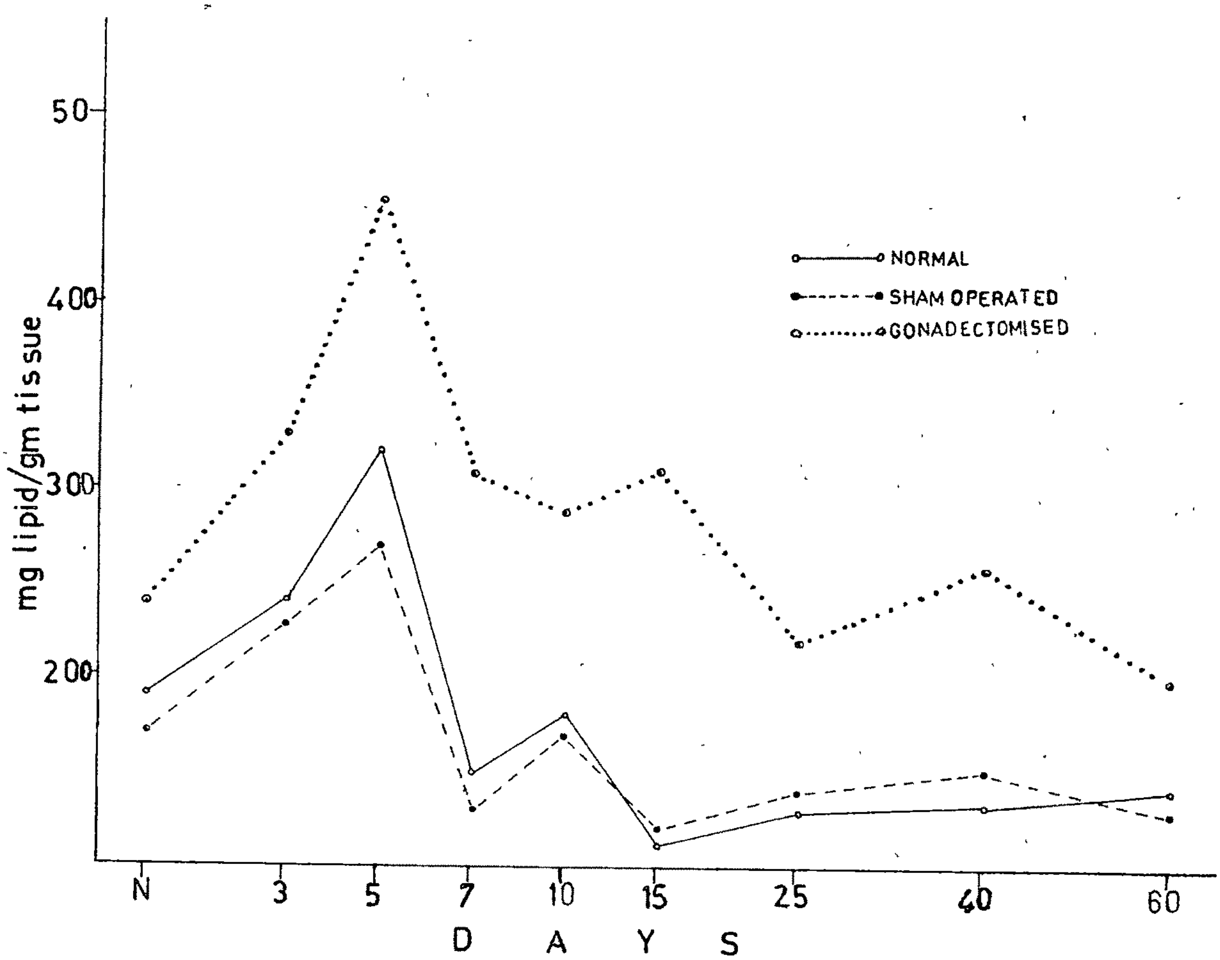

Fig. 1 : Graphic representation of the levels of total lipids in the liver during tail regeneration in the normal, sham operated and gonadectomised lizards, <u>H. flaviviridis</u>

Table 2. Changes in total caudal lipid contents (mg/gm 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)	87.2 ±1.8	156.3 ±4.32	189.1 ± 1.86	148.2 ±3.73	121.4 ±2.25	106.3 ±3.67	119.5 ±3.01 $<0.0005^{@}$	158.9 ± 1.36 $<0.0005^{@}$	108.4 ± 2.39
Sham operated (SGX)	92.3 ±4.03 $<0.0005^{@}$	159.0 ±4.23	106.9 ±2.83	120.4 ± 4.35	97.9 ±3.52	97.3 ± 2.60	120.4 ± 1.17	165.3 ± 4.58 $<0.0005^{@}$	132.1 ± 3.49
Gonadec-tomised (GX)	110.4 ±2.79 $<0.0005^{@}$	280.6 ±9.18	123.0 ±1.98	214.9 ±6.6	266.9 ±5.44 $<0.0005^{*}$	190.8 ±2.47	135.06 ±3.09	194.6 ±4.74 $<0.0005^{*}$	282.4 ±2.21

± S. D.

* P value obtained in comparison with SGX

@ P value obtained in comparison with IC

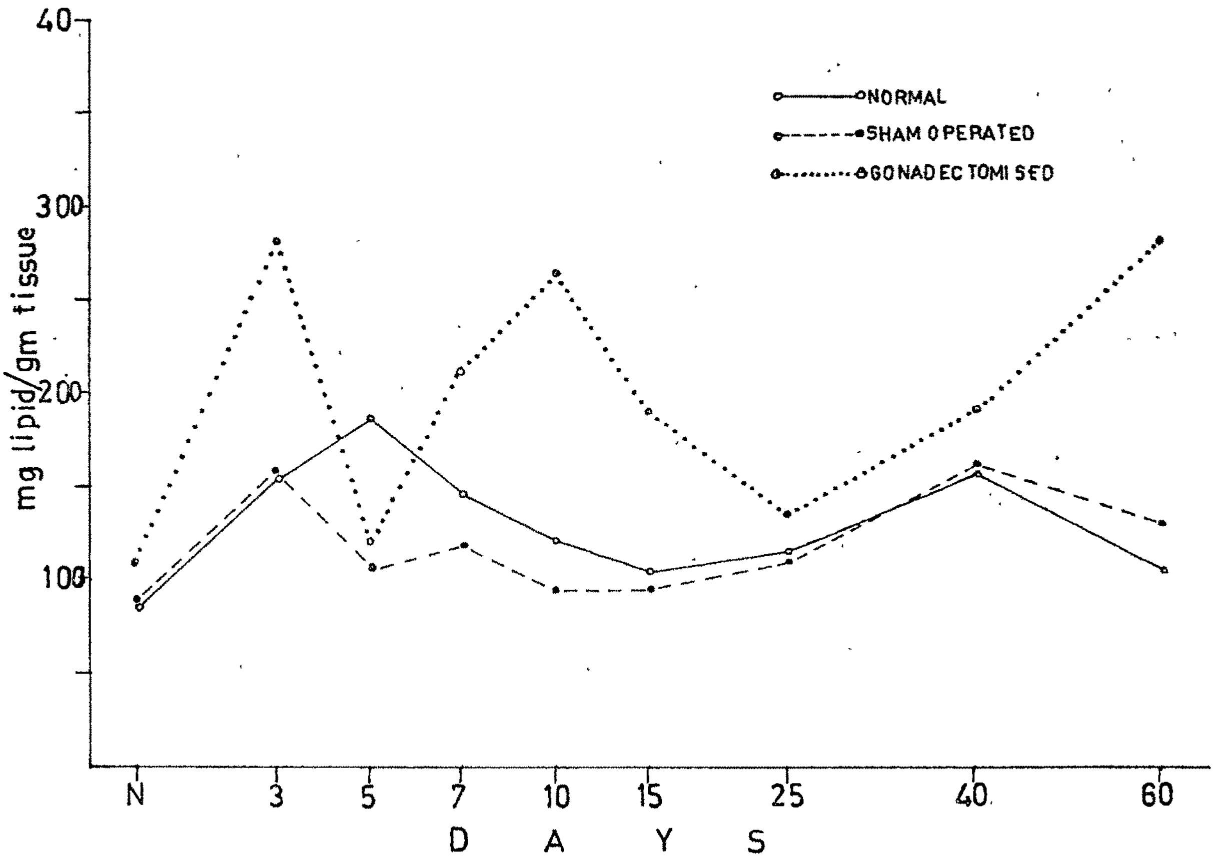

Fig. 2 : Graphic representation of the levels of total lipids in the regenerate during tail regeneration in the normal, sham operated and gonadectomised lizards, *H. flaviviridis*

then continued depleting till the 15th day in the control groups and till the 25th day in the GX group. This level was more or less maintained thereafter till the 60th day. The total lipid content of the tail of normal and the SGX lizards depicted a similar pattern with very little difference. The GX animals showed maximum oscillations. The raised level of lipid noticed prior to autotomy increased on the 3rd day followed by a fall on the 5th day. Thereafter, there was another increase to a higher level by 10th day followed by a steady decline till the 25th day and a rise to a high level through the 40th to the 60th days. In the case of intact and sham controls, the lipid content increased during the first 5 days and then decreased gradually till the 25th day. Thereafter on day 40 there was another increment which ultimately settled to the pre-autotomy level by the 60th day (Figures 1 and 2; Tables 1 and 2).

Phospholipids : Pre-autotomy level of caudal phospholipid was slightly elevated in SGX, and decreased in GX as compared to the intact controls. First 5 days post-autotomy were marked by tremendously elevated phospholipid content in both the intact and sham controls. After a sudden fall on the 7th day the content again increased to the maximal level by the 25th day of tail regeneration, which then gradually

Table 3. Hepatic and caudal phospholipid contents (mg/gm 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
Liver									
Normal intact (IC)	12.98 ±1.01	11.322 ±1.82	2.795 ±0.93	5.737 ±0.98	5.138 ±0.832	3.425 ±0.48	8.0 ±1.32	9.33 ±2.11	7.29 ±1.44
Sham operated (SGX)	7.55 ±0.66 0.001@	11.407 ±2.45	3.51 ±1.22	9.425 ±2.26	6.705 ±1.173	4.693 ±0.72	8.519 ±0.99	8.97 ±1.68	8.34 ±0.97
Gonadectomised (GX)	8.898 ±2.43 0.0005@	9.873 ±3.10	16.31 ±4.48 $<$0.0005*	11.45 ±3.34	11.727 ±2.27 0.001*	11.585 ±3.99 $<$0.0005*	6.393 ±1.42 0.0025*	5.6 ±1.33	9.77 ±2.27
Tail									
Normal intact (IC)	3.4 ±0.09	6.49 ±1.42	8.28 ±2.25	5.215 ±1.91	10.214 ±2.36	17.24 ±4.98	21.47 ±6.63	12.245 ±3.15	4.64 ±0.79
Sham operated (SGX)	4.54 ±0.64	6.71 ±1.55	8.26 ±2.66	5.58 ±1.57	12.084 ±4.03	18.64 ±5.07	19.84 ±6.18	13.17 ±3.42	4.59 ±0.79
Gonadectomised (GX)	2.856 ±0.18	5.983 ±1.43	7.49 ±1.77	9.87 ±2.34 0.001*	11.75 ±4.10	15.2 ±5.21 0.001*	18.55 ±3.77 0.0025*	11.25 ±2.62	5.63 ±0.67

± S. D.
* P value obtained in comparison with SGX @ P value obtained in comparison with IC

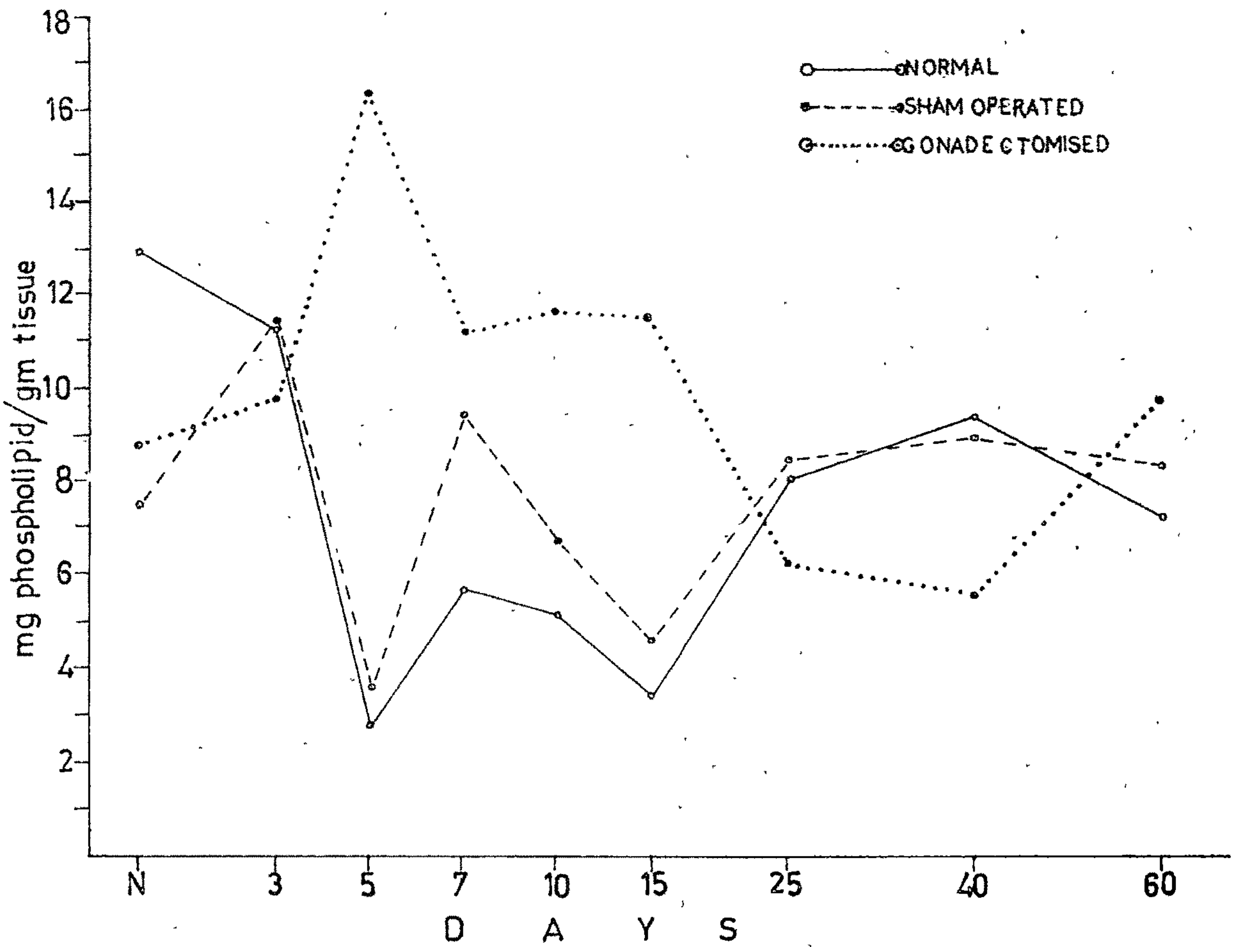

Fig. 3 : Graphic representation of the levels of phospholipids in the liver during tail regeneration in the normal, sham operated and gonadectomised lizards, H. *flaviviridis*.

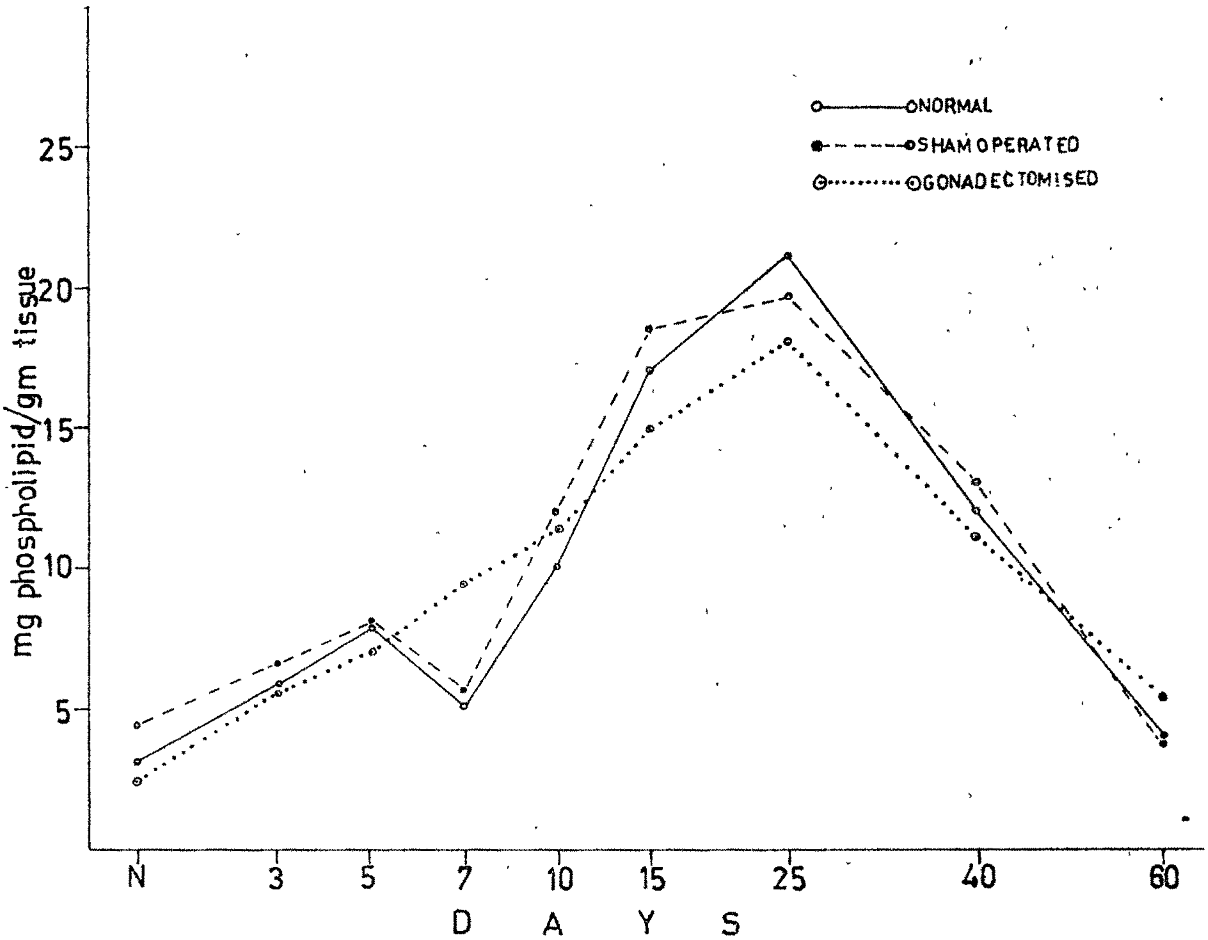

Fig. 4 : Graphic representation of the levels of phospholipid in the tail during its regeneration in the normal, sham operated and gonadectomised lizards, H. flaviviridis

dropped to the normal levels by the 60th day. In contrast, GX lizards showed a continuous increase till the 25th day (Maximal level) and then a decrease to the normal range through 40th to 60th day. Phospholipids of liver were decreased in both SGX and GX lizards in the pre-autotomy condition. Hepatic phospholipids depicted a continuous loss till the 5th day in intact control lizards whence the minimal level was registered. Similar drop was recorded by the SGX lizards also, but with an initial increase on the 3rd day. On day 7, both intact and sham controls showed an increased content which gradually depleted for the second time till the 15th day. Thereafter on days 25th, 40th and 60th, there was an elevated and more or less steady, nevertheless, below normal pre-autotomy level of phospholipid in both the groups. Again, in GX lizards unlike in the controls, the hepatic phospholipid showed an increase till the 5th day whereafter the phospholipid content showed two successive drops, with an initial one on the 7th day (this level remained steady till 15th day) and the second one on the 25th day, which ultimately by 60th day rose to a level comparable to the control groups (Figures 3 and 4; Table 3).

<u>Cholesterol</u> : Pre-autotomy level of hepatic cholesterol was minimal in SGX lizards and intermediate in GX ones as compared to the normal controls. However, the caudal

Table 4. Hepatic and caudal cholesterol contents (mg/gm 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
Liver									
Normal intact (IC)	10.766 ±3.11	9.02 ±2.03	9.65 ±3.54	5.99 ±0.88	8.002 ±1.73	3.727 ±0.57	5.23 ±1.50	4.976 ±1.05	10.08 ±2.72
Sham operated (SGX)	7.84 ±1.63	3.648 ±1.14	6.485 ±2.04	5.494 ±1.07	3.866 ±0.77	7.505 ±2.015	7.79 ±2.45	6.222 ±0.97	9.69 ±2.22
Gonadec-tomised (GX)	9.235 ±2.77 0.001*	11.9175 ±3.48	6.87 ±0.85	8.33 ±2.06	8.502 ±3.13	10.08 ±2.71 <0.0005*	6.48 ±1.82 0.001*	5.58 ±1.53	6.762 ±0.96
Tail									
Normal intact (IC)	3.78 ±0.65	2.18 ±0.78	1.371 ±0.07	5.233 ±1.01	3.12 ±0.18	1.386 ±0.08	1.744 ±0.15	2.734 ±0.001	6.939 ±0.19
Sham operated (SGX)	3.907 ±0.43	2.542 ±0.11	3.33 ±1.13	4.315 ±0.18	1.558 ±0.007	3.847 ±1.45	4.28 ±1.33	3.927 ±0.05	8.312 ±1.94
Gonadec-tomised (GX)	4.101 ±0.92	7.888 ±2.29	5.523 ±1.78	8.817 ±3.11	5.499 ±0.053	9.77 ±1.44 <0.0005*	9.029 ±2.45 <0.0005*	3.877 ±0.66	8.965 ±2.05

± S. D.

* P value obtained in comparison with SGX

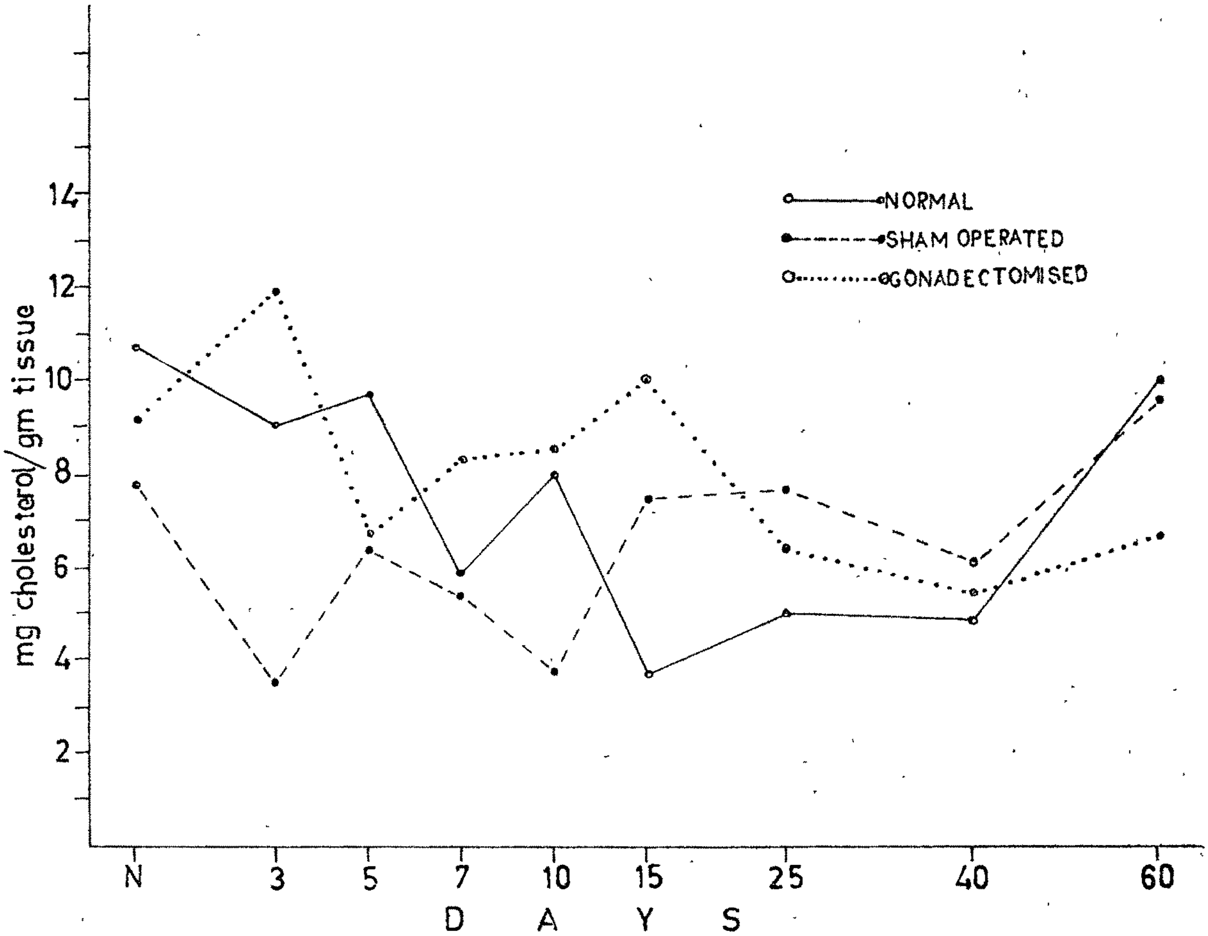

Fig. 5 : Graphic representation of the levels of cholesterol in the liver during tail regeneration in the normal, sham operated and gonadectomised lizards, H. flaviviridis

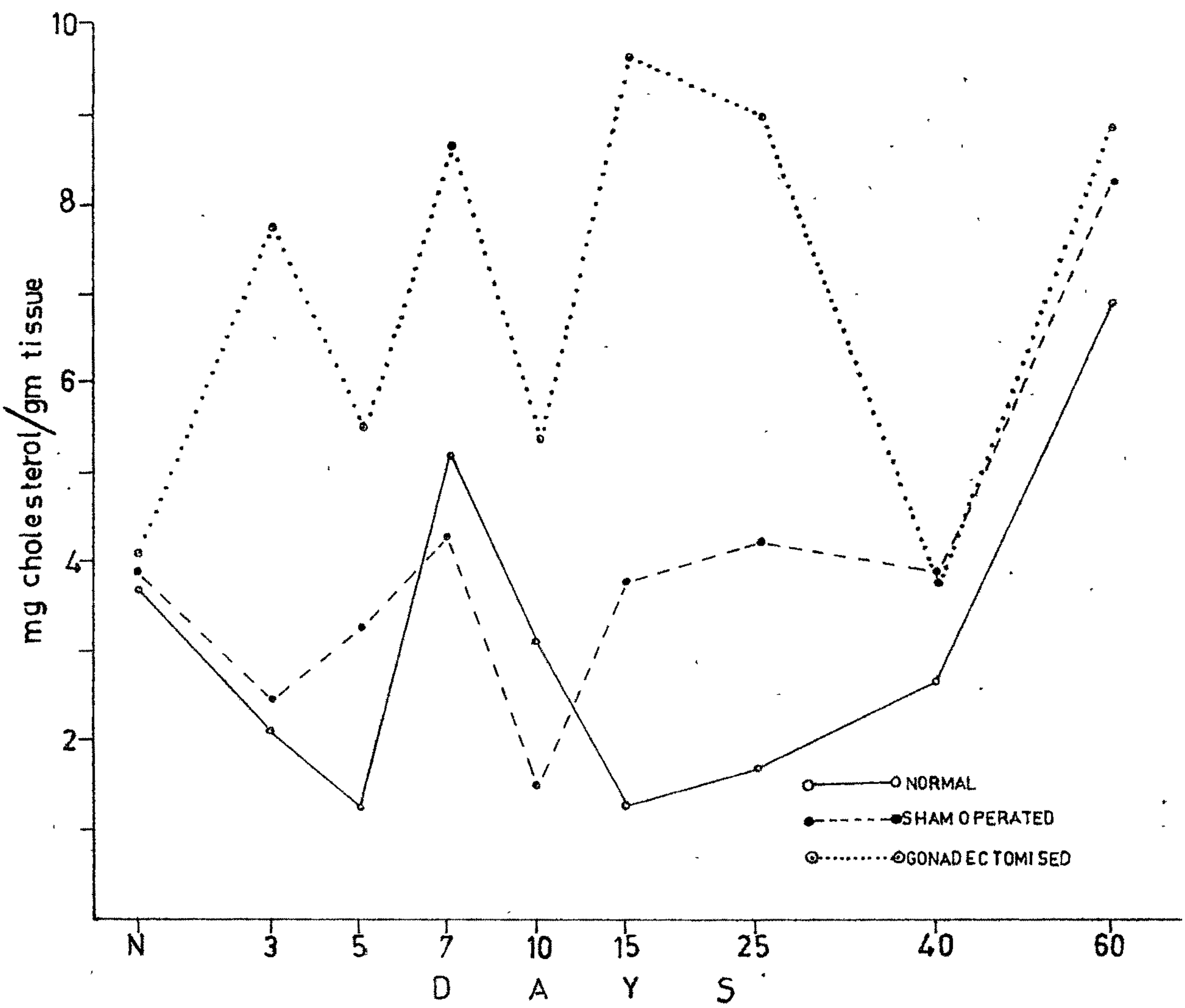

Fig. 6: Graphic representation of the levels of cholesterol in the tail during its regeneration in the normal, sham operated and gonadectomised lizards, *H. flaviviridis*.

cholesterol content was comparable in normal and sham controls while it was slightly elevated in the GX group. Depletion of cholesterol subsequent to autotomy was the feature in both tail and liver by 5th and 7th days respectively in the normal controls. By the 7th day in tail, and 10th day in liver, cholesterol content had increased and thereafter in both the tissues, it remained reduced below normal till the 40th day. By day 60, however, the levels had increased towards the normal range. In the sham operated lizards, hepatic and caudal cholesterol contents depicted continuous oscillations with two noticeable drops, one during the 3rd day and the second one during the 10th day post-autotomy. In contrast, the GX lizards though depicting similar fluctuations as the SGX, however, showed an increase on the 3rd day post-autotomy. Thereafter, though showing fluctuations, caudal cholesterol content was however, elevated during the 15th day. Interestingly at the end of regeneration, hepatic cholesterol content was very much above normal in all the three groups (Figures 5 and 6; Table 4).

<u>Triglycerides</u> : In general, the pattern of alterations of triglyceride content in tail and liver of all the three groups of lizards during regeneration was more or less identical except for minor deviations in GX lizards.

Table 5. Hepatic and caudal triglyceride content (mg/gm 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
Tail									
Normal intact (IC)	80.02 ±10.08	147.63 ±32.04	179.45 ±15.64	137.75 ±28.37	108.006 ±19.97	87.674 ±18.16	96.26 ±15.24	143.92 ±16.68	96.82 ±17.03
Sham operated (SGX)	83.583 ±17.58	149.748 ±29.34	155.31 ±38.35	110.505 ±25.48	84.258 ±11.43	74.813 ±15.61	96.28 ±18.91	148.2 ±30.04	119.197 ±27.03
Gonadectomised (GX)	103.443 ±21.44 0.0025*	266.734 ±39.68	113.34 ±17.58	196.313 ±42.01	249.65 ±27.08	165.83 ±17.38 < 0.0005*	107.48 ± 9.06 0.0025*	179.473 ±24.67	267.715 ±38.41
Liver									
Normal intact (IC)	170.75 ±38.48	221.658 ±30.45	308.455 ±26.07	143.873 ±25.83	172.16 ±30.07	109.348 ±10.93	120.77 ±26.05	122.594 ±27.77	127.63 ±31.85
Sham operated (SGX)	157.91 ±42.31 < 0.0005@	216.445 ±39.57	266.696 ±23.36	117.87 ±19.34	163.299 ±23.55	107.802 ±13.010	117.69 ±20.18	139.80 ±15.37	112.3 ±21.01
Gonadectomised (GX)	231.367 ±33.03 < 0.0005*	311.207 ±48.05	456.42 ±58.62	290.22 ±15.39	277.77 ±32.58	295.635 ±38.44 < 0.0005*	209.427 ±37.10 < 0.0005*	250.22 ±33.92	191.55 ±17.07

± S. D.
@ P value obtained in comparison with IC
* P value obtained in comparison with SGX

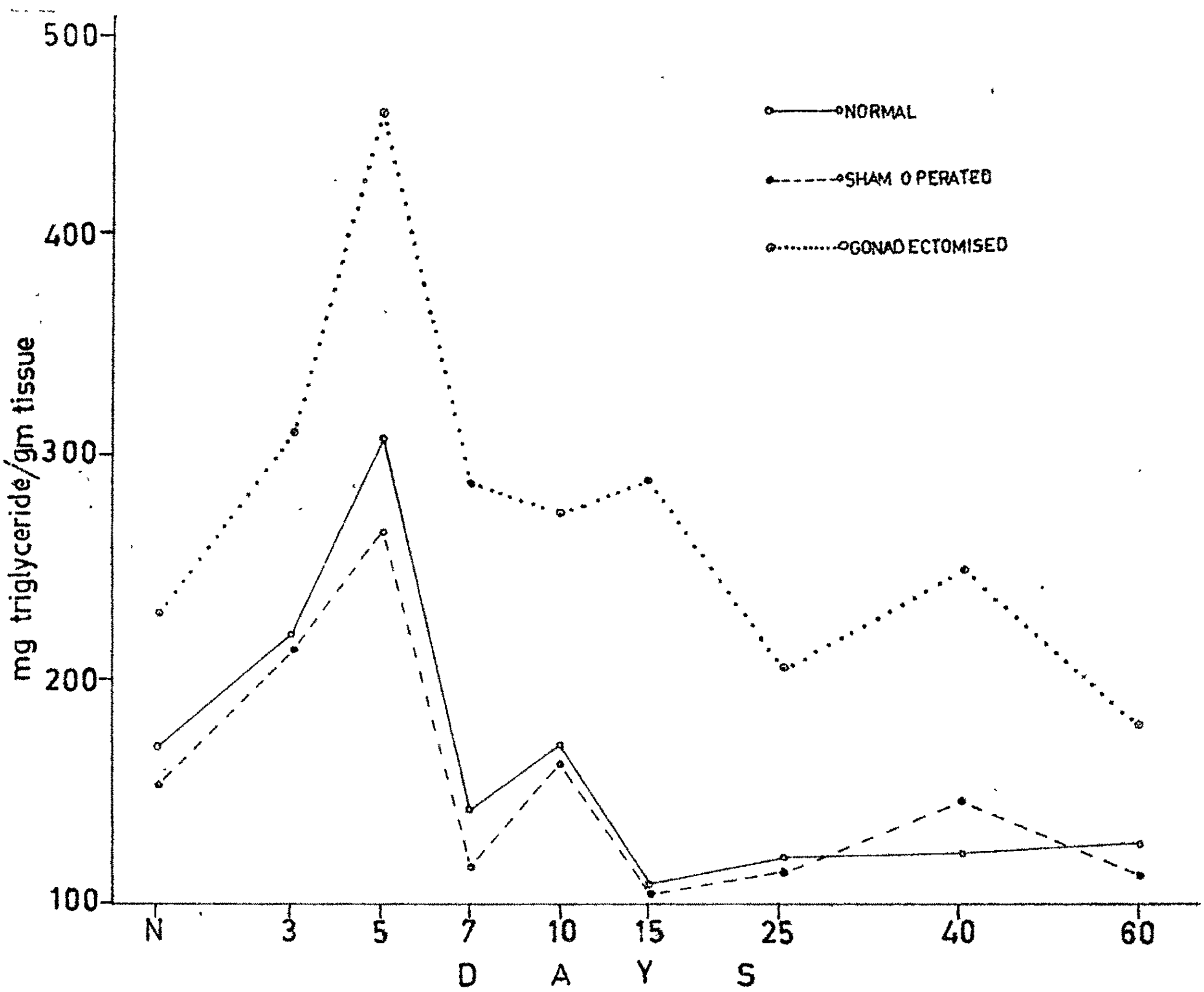

Fig. 7 : Graphic representation of the levels of Triglycerides in the liver during tail regeneration in the normal, sham operated and gonadectomised lizard , *H. flaviviridis*

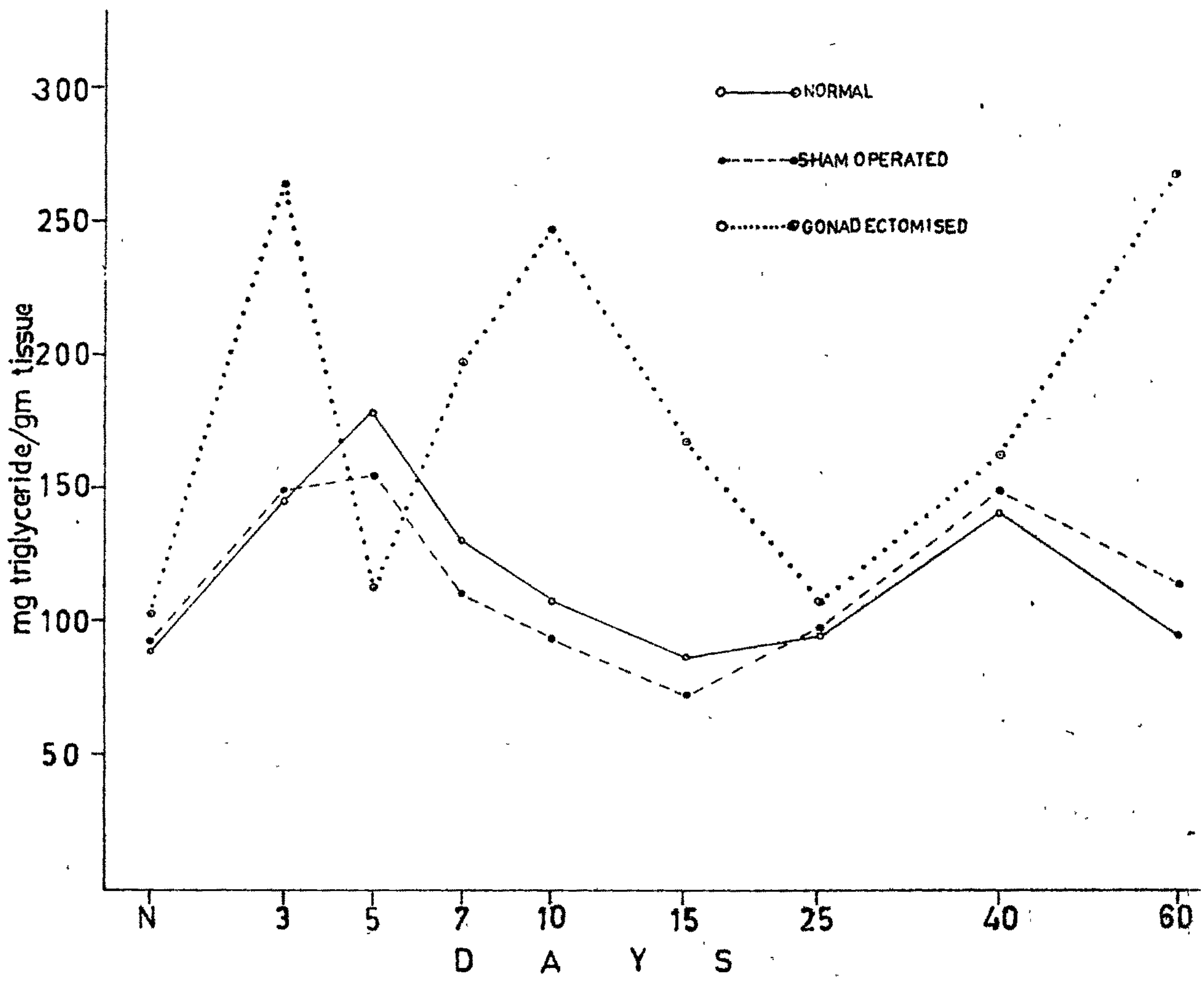

Fig. 8 : Graphic representation of the levels of triglyceride in the tail during its regeneration in the normal, sham operated and gonadectomised lizards, H. flaviviridis

Accordingly, both normal and SGX lizards showed tremendous and continuous increase post-autotomy till the 5th day. Through 7th till 25th day, there was a continuous depletion in caudal triglyceride content, while hepatic triglyceride showed decreased value on the 7th day, an increase on the 10th day and then continued fluctuating below this level, till the end of regeneration. Post-autotomy increase was evident in GX lizards also, with the increment extending into the 5th day in liver and only upto the 3rd day in tail. On day 5th, caudal triglyceride content decreased and then increased on 7th and 10th days. The triglyceride level decreased during 7th and 10th days and then continued fluctuating below this level. The triglyceride content of liver and tail was, in general, higher than that of the normal and SGX lizards all throughout (Figures 7 and 8; Table 5).

DISCUSSION

Local and systemic involvement of lipids in reptilian regeneration reported previously (Radhakrishnan, 1972; Shah and Chakko, 1968; Kinariwala et al., 1979; Chakko and Mariamma, 1981) apparently find justification in the herein observed in loco and systemic alterations in the lipid fractions in H. flaviviridis. The deduction derived by Shah and Ramachandran (1970; 1972; 1973; 1974; 1975; 1976) based on their histoenzymological studies in the regenerating tail of <u>Mabuya carinata</u> of accumulation and utilization of lipids during the regressive and progressive phases of regeneration respectively, is well supported by the <u>in loco</u> increased total lipid and triglyceride contents during the post-blastemic phase noted presently. Significant hepatic participation is also suggested by the noted parallelism in the temporal pattern of changes as well as the prevalence of below normal levels of both total lipids and triglycerides from the 7th day onwards.

Importance of phospholipids and cholesterol in the regenerating system for the structural layout of dividing and differentiating cells hinted at by Shah and Ramachandran (1975) and Ramachandran <u>et al</u>. (1979), is also reflected in the observed <u>in loco</u> increase of phospholipids as well as cholesterol (Tables 3-6; Figures 3-6). Once again, an influence on systemic profile is denoted by the alterations in hepatic phospholipid and cholesterol fractions. A cursory comparison of systemic response in terms of lipid metabolism between <u>M</u>. <u>carinata</u> and <u>H</u>. <u>flaviviridis</u> tends to denote the latter to rely principally on hepatic lipids as opposed to the dependence of the former on viseral fat bodies (Shah <u>et al</u>., 1982), and is in accordance with the reported relative insensitiveness of the fat bodies in <u>H.flaviviridis</u>

and of the hepatic tissue in *M. carinata* (Shah *et al.*, 1977; Kinariwala *et al.*, 1978).

An added dimension in the present study is the evaluation of the influence of gonadal hormones in lipid metabolism in relation to tail regeneration. Apparently, gonadectomy does not have any influence on the normal pattern of alterations in *in loco* and systemic lipid fractions outlined above. Except for certain temporal alterations in the pattern of oscillations, there was however, not much difference between GX and control groups of lizards. The only point of significance is the tremendous depletion of caudal cholesterol content in GX lizards between 25th and 40th days of regeneration. The functional correlation, if any, between this depletion and corresponding retardation in regenerative growth (Chapter 7) can at best be in the realm of speculation. A striking feature of the study, however, is the very obvious tendency of GX lizards to maintain higher levels of all the lipid fractions in the regenerate as well as in the liver. This would indicate a favourable influence on lipid anabolism in the absence of sex steroids. Similar inference is drawn in mammals based on the observation of decreased lipogenesis and increased lipolysis in the presence of gonadal steroids and increased lipogenesis and/or accumulation of lipid components in the gonadectomised condition (Nathaniel and Nathaniel, 1966; Watkin *et al.*, 1972; Molgaard *et al.*, 1980; Van Lenten *et al.*, 1981). The regeneration specific internal milieu seems to have a pronounced depleting effect on hepatic cholesterol in intact lizards, which in GX ones is apparently well resisted (Figure 6).

Based on the observations made in the present study, it may be concluded that the *in loco* and systemic oscillations in lipid parameters induced by the stress of regeneration are independent of the gonadal steroids, and that, like in mammals, absence of gonadal steroids tend to have a favourable influence on synthesis and or accumulation of lipid components, especially triglycerides and cholesterol, in lizards also. Whether this availability of higher titres of lipid fractions could have anything to do with the earlier reported increase in regenerative growth between 7th and 25th days post-autotomy in GX lizards is a poser of relevance.