

CHAPTER - 4

SECTION: I TAIL REGENERATION AND LIPID METABOLISM:
CHANGES IN THE CONTENT OF TOTAL HEPATIC LIPIDS,
GLYCERIDES AND, TOTAL BLOOD LIPIDS IN THE
SCINCID LIZARD, MABUYA CARINATA

Lipids constitute a very important group of organic substances which serves as a major reserve of oxidisable substrates in all living systems. They are the chief stored metabolites and are by now well recognised as the metabolites of choice for long sustained activities in animals, as well as for a variety of other biological phenomena involving high energy requirements. Interestingly, lipids have been reported to play an important role in the molecular ecology of regenerating vertebrate appendages. Previous studies in this light during tail regeneration in the lizards, Mabuya carinata and Hemidactylus flaviviridis had revealed an accumulation of lipids in the regeneration blastema (Radhakrishnan, 1972; Chakko, 1968; Hiradhar, 1972). Shah and Ramachandran, (1973, 1975, 1976), Radhakrishnan, (1972), Shah and Chakko, (1968) and Magon, (1970), based on



their enzymological studies on Mabuya carinata and Hemidactylus flaviviridis, had unequivocally demonstrated the utilization of lipids during the late blastemic and differentiation phases of regeneration. Similar observations with regard to accumulation and utilization of lipids during limb regeneration in urodeles have also been made by the following workers (Salpeter and Singer, 1960; Schmidt, 1962a, 1962c, 1963a, 1963b, 1966a, 1966b, 1966c; Schmidt and Weidman, 1964). Though the necessary enzymatic and metabolic machinery necessary for the elaboration of lipids have been well established in the regeneration blastema in vertebrates (Shah and Ramachandran, 1973, 1974, 1976, Schmidt and Weidman, 1964; Wolfe and Cohen, 1963; Niwelinski, 1960), some sort of systemic involvement in supplementing the efforts of local factors could be considered within the realm of possibilities, as the process of regeneration is a long sustained one lasting upto 75-90 days. In this context the hepatic tissue may be considered to play a role, as it is the main biochemical laboratory in a vertebrate body bringing about innumerable chemical transformations according to the dictates of the requirement of the various parts of the body. Liver is also known to

function as a storehouse for lipids in many of the vertebrates. Moreover, studies on carbohydrate metabolism (Chapter - 3) during regeneration has shown certain specific changes in hepatic glycogen content thus illustrating the importance of the hepatic tissue in the regenerative phenomenon. It was in this background that the present investigations on the changes in the hepatic content of total lipids and glycerides were deemed appropriate. Such an investigation would not only help in understanding the extent of direct involvement of hepatic lipids in the regenerative mechanics, but also possibly help in understanding the indirect mode of involvement as could be evidenced by the association of hepatic lipids with other visceral or systemic factors also in operation during regeneration. To have a better understanding of this aspect, a quantitative evaluation of total lipids in blood too was carried out.

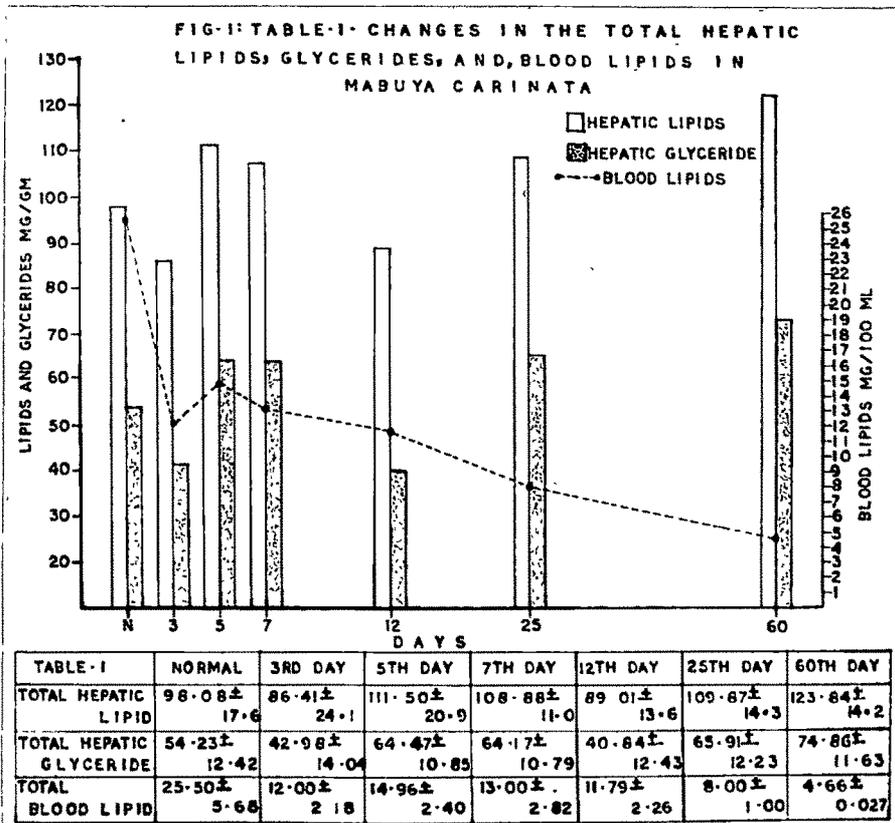
MATERIAL AND METHODS

Adult Mabuya obtained from Mysore, Karnataka State, India, and maintained on a diet of insects

were used as the experimental animals. The animals were allowed a fortnight of acclimatization to the laboratory conditions prior to tail autotomy. The normal as well as lizards with regenerating tails were sacrificed (as per the periods fixed for the current course of study). Liver was collected from the sacrificed animals after blotting them dry of blood and other tissue fluids, and was used to estimate total lipid content by the gravimetric method of Folch et al. (1957). The values of total glyceride content were obtained by deducting the total phospholipid and total cholesterol from the gravimetrically determined total lipid content (Reddy et al., 1972). For the evaluation of total blood lipids, blood samples were collected by cardiac puncture and estimated by the method of Folch et al. (1957).

RESULTS

The quantitative data on total lipids, glycerides and blood lipids are represented in Table-1, Figure-1. A cursory glance of these reveals that the total liver lipids falls below the normal level



(98.08 mg/gm of tissue) once during the early wound healing period (3rd day; 89/41 gm) and once during the late blastema/early differentiation phase (12th day; 89.01 mg/gm). During the other periods, the lipid levels tends to remain above normal with a maximum level (123.84 mg/gm) being attained towards the termination of the process of regeneration. These changes in total lipids in the liver are exactly paralleled by those of glycerides. In contrast, the blood lipid level was seen to fall to a below normal level by the 3rd day itself, which was maintained so more or less upto the 12th day of regeneration. But by 25th day, the level was noted to show a further fall and by day 60 the fall was still more pronounced and reached the ultimate lowest value.

DISCUSSION

The investigations have depicted a wavy pattern of changes as far as the level of hepatic lipid and glyceride contents are concerned during tail regeneration. The most interesting feature is the regular cyclic pattern of depletion and replenishment of the hepatic

stores of these metabolites during the process of regeneration. Such a cyclic course of events may be construed as the repercussions of the varying vagaries of the regenerating system, mediated by the varying titres of the endocrine secretions. In this context a stagewise analysis of these changes would be more informative and useful.

Subsequent to autotomy on the 3rd day, the hepatic lipid content is noted to show a decrease which is almost paralleled by a corresponding identical decline in the glyceride content. This may be construed to indicate that the glycerides from liver are being either released, or, are being utilized within the hepatic tissue itself. Besides, the level of lipids in blood too showed a decline at this period. In this context the most plausible explanation would be of an increased uptake from the blood by the various tissues of the animal including the tail. Even a possible utilization by the liver itself cannot be overlooked as the development of the lymphocytopoietic nodules commences by about this time (Chapter - 2). This phase of hepatic lipid depletion is, however, soon reversed and the lipid as

well as the glyceride levels are noted to show upward trend, with the levels touching above normal values on the 5th day. The increase in the total lipid content in the light of an increase of both phospholipids as well as cholesterol, (Chapter - 5) is rather indicative of an enhanced lipogenesis especially of the glycerides. Possibility of lipid transport from fat bodies to liver also cannot be overlooked, as the fat bodies showed a fall in the percentage weight as well as a definite visible shrinkage in their size (Chapter - 6). This, when viewed in the light of a still subnormal level of blood lipids, is indicative of the fact, that, the lipids are still being steadily withdrawn from circulation at an enhanced rate. The possible utilization of lipid moieties by the immature circulating blood cells for the synthesis of haemoglobin as well as haemoglobin^{transition} (Chapters- 1 & 2) is within the realm of possibility.

With the pregression of regeneration through the blastemic to the early differentiation phase, the hepatic lipid content dwindles steadily through, 7th to the 12th day; whence once again a subnormal level

is registered. It is apparent from the Table - 1; Figure - 1; that the fall in lipid content observed on the 7th day is not however, contributed by that of the glycerides, as the level of the glycerides remains unchanged from that on the 5th day. Hence, the decline may be due to a fall in other lipid fractions (Chapter - 5). But, the pronounced decrease in the lipid content observed during the period between the 7th and 12th days (corresponding to the formation of a regeneration blastema) is paralleled by a simultaneous decline in the glyceride content. A retrospective introspection at this stage reveals the fact, that, the tail regenerate at this stage (blastema phase) is marked by the accumulation of high content of lipid moieties (Radhakrishnan, 1972; Chakko, 1968; Hiradhar, 1972). Schmidt (1966b) based on his tests had characterised the lipids of the regeneration blastema in urodeles as consisting of fatty acids, phosphatides and mono, di, and triglycerides. These observations when reflected on to the present picture obtained on hepatic lipid contents, give a definite indication of an involvement of hepatic

lipids in the regenerative mechanics. This period of lipid loss from liver is also marked by the release of mature lymphocytes from the lymphocytopoietic nodules in the liver in Mabuya; and since a function of lipid transport has been ascribed to lymphocytes during migration in *Rosy pastor* (Pilo, 1970), a similar possibility of lipid transport by the lymphocytes in the present case too could be considered feasible.

With the onset of differentiation in the regenerating tail, the hepatic lipid content traces a course of recovery and, by 25th day itself, the level has reached an above normal one, which by 60th day (whence the regenerate has already progressed to a near fully regenerate condition), attains an all time high level of 123.840 mg/gm. Glyceride fraction appears to provide the bulk of this increase in total lipids. Such a condition of steatosis may be attributed as due to either (a) of an increased lipogenesis (b) decreased uptake from blood, or (c) a transport from depots. The latter two possibilities can be discarded in the light of the currently obtained lowest levels of

blood lipid content during the 25th and 60th days of regeneration, and the corresponding accelerated increase in fat content of the visceral fat bodies (chapter - 6). In this context, the most plausible explanation appears to be a sort of "rebound phenomenon" whereby a fall in lipid content appears to trigger off an increased phase of lipogenesis, and, in this accelerated process of lipogenesis, an enhanced hepatic steatosis appears to be the result.

During the whole course of regeneration, the lipid level in blood was found to be subnormal. An initial fall which was evidenced on the third day itself after autotomy was maintained more or less in the same level till day 12 of regeneration. However, on days 25 and 60 after autotomy, the lipid content was noted to show further decrease. This picture on blood lipid could be taken to indicate a continuous and swift withdrawal of lipid moieties from blood by the various tissues, including the regenerate, in response to their varying physiological necessities during regenerative activities. Finally, it may be mentioned at this juncture, that,

Procaccini et al. (1973) based on their study on changes in liver metabolites had reported an insensitivity of liver lipids during forelimb regeneration in the adult Newt, D. viridescens and had opined that the mechanisms of amphibian fat metabolism are not clearly understood, and supported the suggestion of Brown (1964) that these animals may not degrade lipids during periods of stress. This is substantiated by the observation of Procaccini and Doyle (1968) that the lipid reserves were not depleted even after 12 weeks of starvation in Bufo americana, and, a subspecies of Rana pipens. Viewed in this light, the changes observed in the present study would indicate a clearcut difference between the amphibians and reptiles as far as their metabolic adaptations to regenerative process are concerned.

SECTION: II TAIL WOUND HEALING AND LIPID METABOLISM:
CHANGES IN THE CONTENT OF TOTAL HEPATIC LIPIDS,
GLYCERIDES AND, TOTAL BLOOD LIPIDS IN THE
AGAMID LIZARD, CAIOTES VERSICOLOR

Involvement of hepatic lipids and blood lipids in the regenerative process during tail regeneration in the lizard, Mabuya carinata have been illustrated in the previous section of this chapter. The role of lipids in the repair processes of vertebrates in general is inadequate. Masoro (1960) has reported a general lipemia subsequent to infliction of injury to a mammal which has been determined as a rise in neutral fat, with lesser increase in phospholipids and cholesterol in the plasma (Selye, 1950). It was also suggested that changes occur regardless of the kind of injury inflicted, may it be of heat, crushing, radiation or toriiquet ischemia. Marked increase in plasma lipoprotein too, with an increase in tissue cholesterol were reported by Milch et al.(1954). Apart from these, there are no other reports regarding lipids (involvement) during wound healing and repair

processes in vertebrates. Though a few reports (however meagre) as mentioned above are available on mammals, studies of a comparable nature in reptiles are totally nonexistent. The sparseness of information regarding the metabolic aspects in the repair processes of vertebrates in general and reptiles in particular has stimulated the present study on hepatic content of lipids and glycerides, and, blood lipids during the healing of the tail wound after amputation in *Calotes*. Moreover, such a study could give a comparative idea on these aspects between the regenerative and nonregenerative types of wound closure in lizards and possibly also help in confirming the changes observed in *Mabuya* represented in the previous section of this chapter on these very aspects, as specific responses elicited essentially due to regeneration. In this context, a quantitative evaluation of total hepatic lipids, glycerides and, blood lipids is attempted in the Agamid lizard, *Calotes versicolor*.

MATERIALS AND METHODS

The adult *Calotes* obtained from the local animal

dealer and maintained on a diet on insects were used for the experiment. The animals were allowed a fortnight of acclimatization to the laboratory conditions, and, the tails of these lizards were then amputated with a sharp scalpel. The cut end of the tails were kept pressed with the fingers for a minutes to prevent excessive blood loss.

The estimation of hepatic lipid and glyceride contents, and, blood lipid during the various periods of wound healing as well as in the normal case (nonamputated) were assayed by the methods mentioned in the previous section.

RESULTS

TOTAL HEPATIC LIPIDS AND GLYCERIDES

Significant and very pronounced changes in the content of total hepatic lipids and glycerides appeared to be the feature of the normal wound healing in the lizard Calotes versicolor. The average normal level of hepatic lipids and glycerides were 104.810mg/gm and 34.864mg/gm respectively. On the 3rd day after tail amputation, lipid as well as glyceride contents

FIG-1: TABLE-1. CHANGES IN THE TOTAL HEPATIC LIPIDS, GLYCERIDES, AND BLOOD LIPIDS IN CALOTES VERSICOLOR

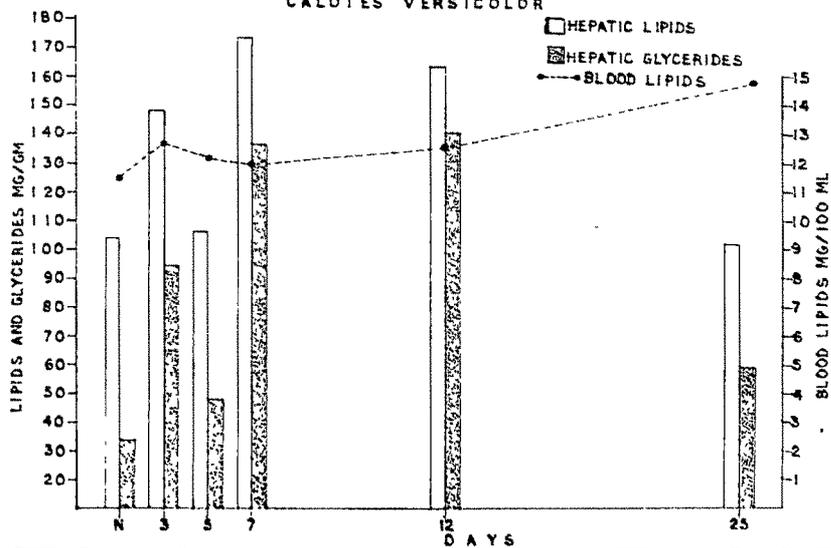


TABLE-1	NORMAL	3RD DAY	5TH DAY	7TH DAY	12TH DAY	25TH DAY
TOTAL HEPATIC LIPID	104.81± 13.62	148.85± 15.49	106.36± 15.26	173.30± 17.75	163.60± 14.22	102.46± 14.75
TOTAL HEPATIC GLYCERIDE	34.86± 12.38	94.30± 15.28	48.83± 14.34	136.75± 17.14	140.34± 13.84	59.69± 14.41
TOTAL BLOOD LIPID	11.50± 2.15	12.60± 2.32	12.10± 2.24	11.90± 2.01	12.50± 2.48	14.80± 2.81

showed a significant increase only to return to a more or less normal (though slightly higher than normal) level on the 5th day. However, by the 7th day the hepatic lipid and glyceride contents increased again and reached the maximum level of 173.300mg/gm and 136.757mg/gm respectively.

Thereafter, there was a progressive depletion of lipids through 12th to the 25th day at which time, their levels had more or less attained normalcy. These changes in the hepatic lipid and glyceride fractions are depicted in Table - 1; Figure - 1. Though such significant fluctuations were observed with regard to hepatic lipids and glycerides, however, as could be seen from the table and figure, the total blood lipid level appeared to remain more or less unchanged all throughout the course of the present study.

DISCUSSION

The changes in the content of hepatic lipids and glycerides observed in the present course of study during tail wound healing appears to be more or less of a diametrically opposite pattern when

compared with the changes occurring during tail regeneration in Mabuya. This possibly illustrates the differential systemic response to the regenerating and nonregenerating systems in reptiles, and also possibly highlights the adaptation of the animal's body endowed with the power of regeneration in relation to its regenerative ability. The immediate change subsequent to tail amputation on the 3rd day in the case of Calotes is an apparent hepatic steatosis marked by an increased content of total lipids and glycerides. Such an increase of lipid at this period accompanied by its slight increase in the blood as well, might signify the mobilization of lipids from other sources and/or synthesis within the liver itself. The observation of a significant hepatic protein depletion at this period (Chapter - 7) could be of some interest in this connection, and, possibly underscores the functional interrelationship between proteins and lipids. The changes observed (of a reduction of hepatic lipid and glyceride contents) in the case of Mabuya at this period after tail autotomy appears to be in this light, of a more specific nature

invoked to meet the exigencies of the regenerative mechanics. However, in the present case too (in Calotes) as noted for Mabuya, the trend of changes observed on the 3rd day gets reversed on the 5th day, and is marked in Calotes by a fall in hepatic lipid content which is paralleled by a corresponding fall in the glyceride content as well. Though the functional significance of this fall in lipid content is rather vague, it may however, be explained on the basis of an increased utility value in energy yielding reactions associated with the healing process. This may appear more tenable in the light of the relative insensitivity of the hepatic glycogen to the amputational stress in the case of Calotes (chapter - 3). Another distinct possibility is the supply of lipid moieties by liver to other organs, especially the haemopoietic ones, as there is a fall in blood cell count to half its normal value at this period to be immediately followed by an elevated above normal value on the 7th day (chapter - 1), and the appearance of abnormal lipids in spleen (Unpublished data). A concomitant increase in the hepatic protein content (to an almost normal level; chapter - 7) is again interesting.

The most interesting feature of the present study is the tremendous increase in the hepatic lipid content (Table - 1; Figure - 1) noticeable on the 7th and 12th days postamputation. An increase to such an extent could be considered as a case of steatosis. The corresponding histological observations of liver sections made during these periods indicate the development of "fatty liver" as denoted by the presence of many lipid laden cells, very much comparable to the adipocytes. Such a condition of "fatty liver" has been associated with wound healing of liver in diabetic rats (Shah et al., 1974; Kishnani, 1975) and other mammals with other abnormal physiological conditions (Popper and Schaffner, 1957). However, the development of such a condition noted in the present case during the tail wound healing is of common occurrence during wound healing in vertebrates in general, or, is more of a specific nature in the case of reptiles, can neither be resolved nor corroborated at this juncture as there is a dearth of investigations of this nature. The data presented in the Table - 1; Figure - 1, also indicate, that, though there is a slight drop in the total lipid

content on the 12th day the glyceride content remains almost unchanged, and could be attributed as due to a fall in other lipid fractions (chapter - 5). This condition of fatty liver and enhanced steatosis appears to get corrected by about the 25th day as depicted by the lipid and glyceride contents of the liver, whence some semblance of normality in the microarchitecture of the hepatic tissue also is restored. In this process of reverting back to normal, the depletion of hepatic lipids is accompanied by an increase in the blood lipid level which might be an indication of the tremendous rate of lipolysis associated with the process of reversal. Interestingly enough, the fall in hepatic lipid content is once again reciprocated by a corresponding increase in its protein content (chapter - 7).

The fluctuating changes observed in the present study, such as, an initial steatosis (3rd day) succeeded immediately by a depletion, again followed by a more pronounced steatosis and development of fatty liver (7th and 12th days), and, followed by a recoument to the normal condition (25th day) of *the organ*: the functional significance of these and the associated controlling mechanisms all remain completely

enigmatic. Such a complex course of events might, however, be considered to be interlinked with endocrine factors. Detailed studies of this nature would be more informative and possibly would help in understanding the functional significance of the changes during wound healing observed herein.