

CHAPTER - 5

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

The major constituents coming under the class of lipids are the glycerides, phospholipids and cholesterol. Cholesterol is an important sterol, which is widely distributed and is a constituent of all animal cells. It might function as a special transport agent for unsaturated fatty acids in the blood plasma, as a precursor of bile acids in liver, as a precursor of various steroid hormones, as an insulatory material for the nerve fibres in the brain and nervous tissue and, also, as a constituent of a number of types of cell membranes. The phospholipids like sterol are present in all cells and are also known as phosphorized fats, phospholipins or phosphatides. They are constituents of all cells and play vital roles as constituents of cell membranes, and, factors in regulating membrane permeability, and are present in

the myelin sheath of nerve cells, and in electron transport particles. They also serve as biologic detergents aiding in the solubilization of the less polar lipids like triglycerides and cholesterol in the aqueous fluids of the body.

Presence of phosphatides (Schmidt, 1966b), plasmalogens (Hayes, 1949), mitochondrial cephalin, lecithin, and spingomyelin (Baker, 1946) have all been associated with the amphibian regeneration blastema. In the case of reptiles too during tail regeneration, the importance of phospholipids have been hinted at by Shah and Ramachandran (1975) based on their studies on histochemistry of dehydrogenases and that of Radhakrishnan (1973) on the histochemical distribution of lipids.

Moreover, in the previous chapter (section - I) blood lipids, hepatic lipids and glycerides were studied in relation to the regenerative process in *Mabuya*; and the study yielded certain definite pattern of changes which were correlated with the various physiological necessities of the body in the wake of the repair and regenerative mechanics in operation.

To have a clear understanding of the involvement of the hepatic lipids during regeneration, it was thought desirable to monitor the changes in the other lipid fractions too such as phospholipids and cholesterol. Further the levels of blood cholesterol also were assayed.

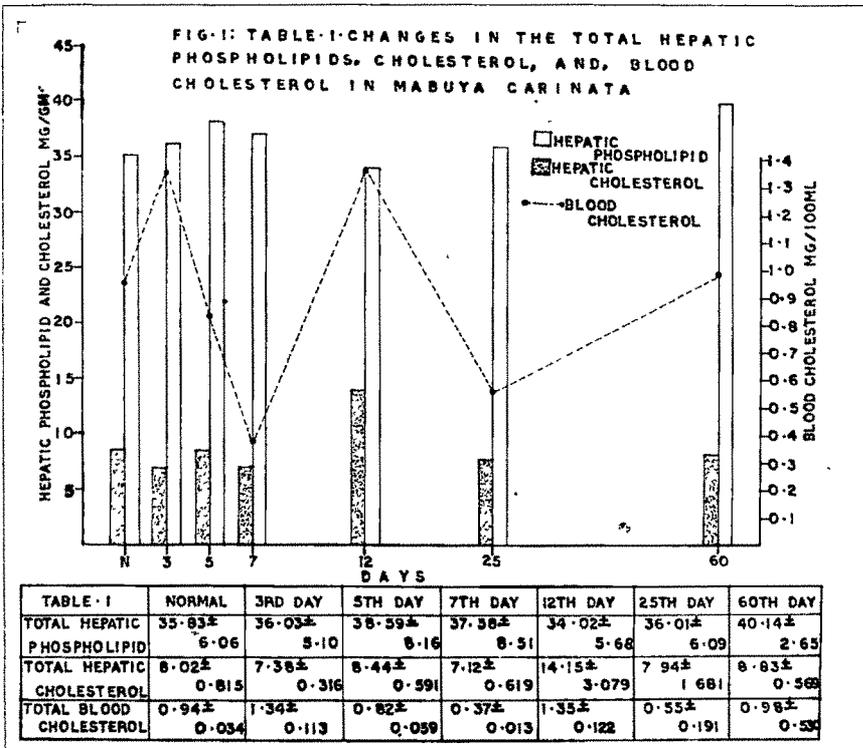
MATERIAL AND METHODS

Adult Mabuyas obtained from Mysore, Karnataka State, India, and maintained on a diet of insects were used as the experimental animals. After allowing sufficient time for acclimatization to the laboratory conditions, the tails of these animals were autotomised and sacrificed as described previously at fixed intervals of 3, 5, 7, 12, 25 and 60 days respectively during tail regeneration. The liver and blood samples from these animals and from normal ones (nonautotomised) were taken and quantitative evaluation of phospholipids and cholesterol was carried out as per the methods of Dittmer and Wells, (1969) and Crawford, (1958) respectively.

RESULTS

HEPATIC PHOSPHOLIPIDS

The changes in the hepatic phospholipid contents



during different periods of tail regeneration in Mabuya are presented in Table - 1; Figure - 1. The liver phospholipid content increased slightly on the 3rd day and more prominently on the 5th. There was a gradual and steady fall of this lipid since then through 7th day and ultimately reached a subnormal level on the 12th day. The phospholipid content once again started increasing and attained a more or less normal level on the 25th day to reach subsequently an all time high level on the 60th day.

HEPATIC CHOLESTEROL

During the tail regeneration the cholesterol content of liver showed an initial fall on the 3rd day and a subsequent increase on the 5th day. This was followed by a further fall on the 7th day only to increase to an above normal level on the 12th day. This high level of cholesterol was seen to come down and touch the normal level on the 25th day and thence on the 60th day the level tended to remain slightly above normal.

BLOOD CHOLESTEROL

In the initial stages of tail regeneration blood

cholesterol was noted to show mirror image pattern of changes (with reference to hepatic cholesterol) thus marking an increase on the 3rd day and a fall on the 5th day. Thereafter the cholesterol content of the blood was seen to depict parallel changes with that of hepatic cholesterol. Thus the blood cholesterol level came down further on the 7th day to be followed by an increase on the 12th day only to show a further fall on the 25th day. On day 60, like hepatic cholesterol, blood cholesterol too was seen to have a tendency to remain at a slightly above normal level. The changes in the hepatic and blood cholesterol levels are presented in Table - 1; Figure - 1.

DISCUSSION

Tail regeneration induced fluctuations in phospholipids and cholesterol in the liver and blood as depicted in Table - 1; Figure - 1, do show certain changes (though not that well pronounced as in the case of total lipids and glycerides) during the progression of regeneration. The hepatic cholesterol and blood cholesterol changes show a mirror image

pattern in the initial stages of tail regeneration lasting upto the 5th day. However, thereafter, the changes remain parallel till day 60. On the other hand, in the case of hepatic phospholipids there is an increase on the 3rd and 5th days. The patterns of changes seen from the 5th day onwards tend to show a parallel course with that of total lipids and glycerides (chapter - 4, section - 1). The increase in the phospholipid content observed on the 3rd and 5th days could be correlated with the lymphocytopoietic activity as well as the infiltration and destruction of red blood cells in the liver during these periods (chapter - 2). Possible entry of phospholipid material into the liver from the cut end of the tail tissues could also be imagined in the light of the observed extensive lipophanerosis of injured tissues, presence of numerous lipid engorged macrophages, and, positive staining response for phospholipids including plasmalogens and cholesterol at the amputation surface of newt limb immediately after amputation (Schmidt, 1968). In contrast, the cholesterol level in liver appears to fall on the 3rd day which might in the light of a general lipid depletion (chapter-4)

be looked upon as an associated change. This is reflected in the form of an elevation of cholesterol content in the blood, towards which, even the degradatory changes associated with injury could also be a contributing factor. In the light of the earlier hindered involvement of thyroid hormone during the early phases of regeneration (chapters- 1 & 3), which is further strengthened by the observation of reduced colloid content in the thyroid gland acini on the 3rd day after autotomy (unpublished data) and, the reported inverse relationship between thyroxine and plasma cholesterol (Kritchevsky, 1964; Masoro, 1968), the decline in the blood cholesterol level observed herein between the 3rd and 5th days postautotomy becomes rather self explanatory.

The periods between 5th and 7th, and 7th and 12th days characterised by the blastema formation, are marked by differential pattern of changes with regard to the three parameters under investigation. Whereas the hepatic phospholipid content dwindled continuously from the 5th, through 7th, to 12th day, the cholesterol content in both the liver and blood, showed, first a decrease on the 7th day and an increase on the 12th

day, with the increase in the hepatic component being much more pronounced. Though the fall in the blood cholesterol value on the 7th day could be explained on the basis of a persisting effective level of thyroxine in circulation; it however, appears to be not that simple, when viewed in the wake of a declining level of cholesterol in the liver as well. Such a state might be indicative of an active utilization of cholesterol by the body which is tuned to meet the specific requirements associated with regenerative process. The subsequent increase in the cholesterol content to a peak level on the 12th day in the liver is indicative of an active biosynthesis of cholesterol, which gets reflected in the form of an elevation of its level in the blood as well. However, its increase in the blood content is not proportionate to the tremendous increase noted in the liver which could be suggestive of its continuous utilization. At the same time, a possible functioning of cholesterol in the transport of unsaturated fatty acids (Orten and Neuhaus, 1970) to the site of regeneration during these periods may be supposed, as fatty acids have been demonstrated in the regeneration blastema of

Xenopus laevis tadpoles (Hess, 1959). Meanwhile, the phospholipid content of the lizard liver was noted to show a continuous fall during these periods till it reached a minimal level on the 12th day. A number of reasons could be extended to explain this fall in hepatic phospholipids. One such possible explanation is, that, this might be correlated with the maturation and release of lymphocytes from lymphocytopoietic nodules as observed at this period (chapter - 2). The other possibilities are, that, it may be closely connected with the general hepatic lipid depletion noted during these periods, or, they are being utilized for forming the serum lipoprotein fractions involved with the transport mechanisms of lipids. Possible participation of phospholipids together with cholesterol in the formation of large quantities of bile that may be required for the effective digestion of food that may be consumed by the animal during this period after the initial injury induced stress (chapters- 3 & 6) might also be a matter of significance. Such an involvement of phospholipids and cholesterol in the synthesis of bile during the arousal period of hibernating little

brown bats, Myotis lucifugus and during bird migration in Sturnus roseus have been suggested by Escher et al. (1973) and John (1967) respectively.

With the onset and progression of differentiation in the regeneration, both, the hepatic as well as blood cholesterol contents came down to subnormal levels on the 25th day, thus indicating an active and fast paced utilization of cholesterol moieties. Some part of it may be expected to be taken up by the differentiating system for its various functional needs. Moreover, this fall could also be indirectly related with the increasing titres of thyroxine that may be released at this phase of regeneration for the control of the processes of division and differentiation of cells. Once again, the observation of a reduced content of colloidal material within the thyroid acini (Unpublished data) appears to lend credence to this suggestion. However, on the corresponding period, the liver phospholipid content is noted to show a continuous and steady increase which towards the near culmination of the regenerative process on the 60th day registers the highest level that has been recorded in the present

study. This behavioural pattern of phospholipids appears to correspond well with the high level of glycerides and total lipids observed in the liver (previous chapter). In this process of steatosis, even the cholesterol content is seen to attain an above normal level by 60th day from a more or less normal level observed on the 25th day. Such a case of steatosis may be due to the prevalence of an anabolic influence which could be corroborated not only with the synthesis of macromolecules within the regenerate, but also with the increase of various biochemical moieties in the body (chapters- 4,5,6,7,9). Some steroids with androgenic principles could be considered to play an important role in this connection, in which case the tremendous depletion of cholesterol in both the liver and blood observed between the 12th and 25th days, could be considered as due to its active incorporation into the biosynthetic machinery of such steroids that may be in operation in liver, adrenal and gonadal tissues. Though such an anabolic environment could be considered effective in raising up the phospholipid content of hepatic tissue, the possible contribution by the large number of red

blood cells that are being destroyed within the liver
as observed on the 25th day (chapter - 2) also
cannot be overruled.

SECTION : II TAIL WOUND HEALING AND LIPID METABOLISM:
CHANGES IN THE CONTENT OF TOTAL HEPATIC CHOLESTEROL,
PHOSPHOLIPIDS, AND, BLOOD CHOLESTEROL IN THE
AGAMID LIZARD, CALOTES VERSICOLOR

In chapter - 4, section II, changes of a very significant nature were recorded with respect to hepatic total lipids, glycerides and blood lipids during tail wound healing in Calotes. These changes were further noted to be of a distinctly different pattern in comparison with those occurring during tail regeneration in Mabuya. Having established such a distinction between the two cases (tail regeneration and tail wound healing) it would be more pertinent to extend the studies on to the other classes of lipids as well, such as phospholipids and cholesterol so that not only the involvement of these factors during normal reptilian wound healing could be ascertained, but also derive a comparative idea thereof between the regenerative and nonregenerative systems. Studies on lipids and their functions in the injury and repair processes are scanty, and, even those few which are

documented are pertaining to the local site of injury itself. Most of these studies using a number of procedures and techniques have all indicated an increase in phospholipid content and decrease in neutral lipids (Schilling and Milch, 1955; White et al., 1959; Boncek and Nobel, 1957; Bole et al., 1962; Levin and Head, 1965; Jackson, 1964; Bole, 1964). It was in this background that a quantitative evaluation of cholesterol in liver and blood, and, phospholipids in liver was undertaken so as to have an idea of the extent of involvement of systemic factors in wound healing and also to confirm the changes observed in the previous section as due to regeneration itself.

MATERIAL AND METHODS

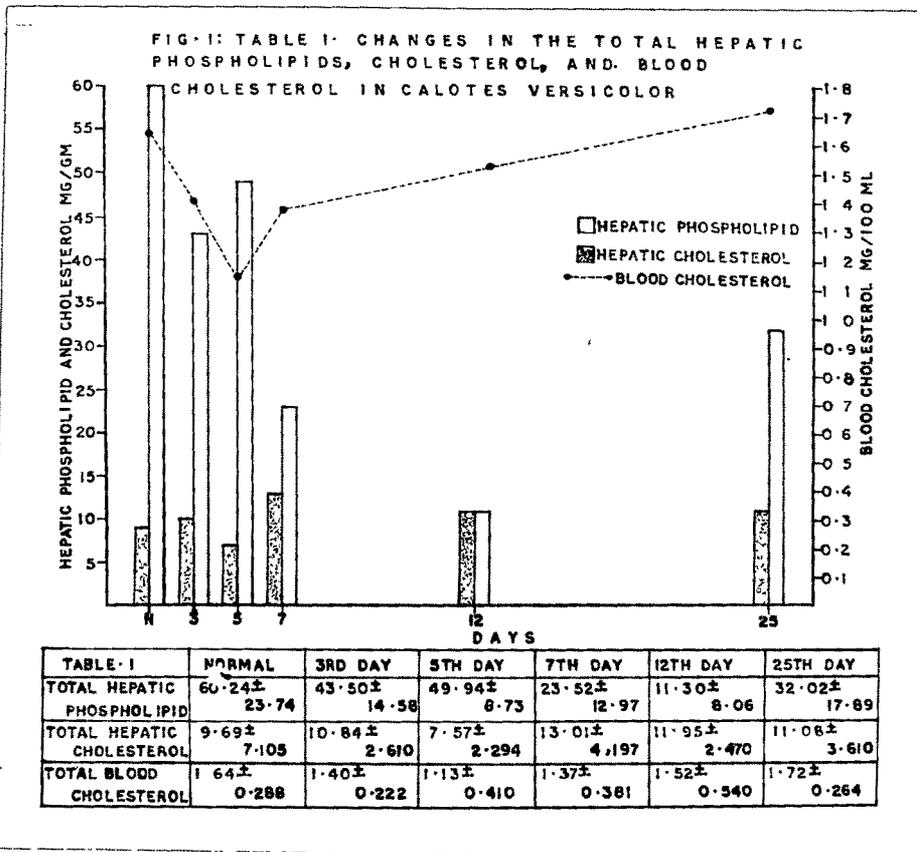
Adult Calotes of the same weight group obtained from the local animal dealer and maintained on a diet of insects were used as the experimental animals. The animals ^{were} allowed a fortnight of acclimatization to the laboratory conditions and then the tails were amputated with a sharp scalpel. The cut end of the tails were kept pressed with fingers for a few minutes

to prevent excessive blood loss and were then sacrificed under mild anaesthesia at the fixed intervals of 3,5,7,12 and 25 days respectively after amputation. The blood and liver samples from these and normal (without amputation) animals were then taken and the content of cholesterol and phospholipids in them assayed by the methods of Crawford (1958) and Dittmer and Wells (1969) respectively.

RESULTS

The changes observed in the content of hepatic phospholipids and cholesterol, and, blood cholesterol are depicted in Table - 1; Figure - 1. The changes in the case of hepatic phospholipids were more spectacular, in that, it showed a continuous depletion from a normal level of 60.24 mg/gm to a very low level of 11.30 mg/gm on the 12th day. Subsequent to this, on the 25th day there was a substantial recovery and attained a level of 32.02 mg/gm.

On the other hand, liver cholesterol level was seen to show a slight increase on the 3rd day (9.69mg - 10.84mg) to be followed by a fall on the 5th day



(7.57mg/gm). This was soon succeeded by an attainment of the highest level (13.01mg/gm) on the 7th day. Though the cholesterol level showed a decline thereafter through the 12th day (11.95mg/gm), the level, however, remained above normal even on the 25th day (11.08mg/gm).

In contrast, the blood cholesterol content showed only two clear cut changes. There was an initial fall from the normal level of 1.64mg/100 ml, to the lowest level of 1.13 mg/100 ml on the 5th day, through an intermediate level of 1.40mg/100 ml on the 3rd day. Thereafter the blood cholesterol level gradually and slowly rose through 1.37mg/100 ml, and, 1.52mg/100 ml, to 1.65mg/100 ml on the 7th, 12th and 25th days postamputation respectively.

DISCUSSION

The clear cut distinction that was observable with regard to the total lipids and glycerides between tail regeneration and mere tail wound healing, is once again revealed even in the case of phospholipids and cholesterol. Whereas during tail regeneration there was a constant increase of hepatic phospholipid

content during the first five days, in the present case however, the phospholipid content declined sharply on the 3rd day and then rose slightly on the 5th day. This when viewed in the light of the hitherto reported (chapter - 4, section II) hepatic steatosis observed on the 3rd day, and a fall on the 5th day, accompanied by corresponding parallel changes in the glyceride content, appears rather self explanatory. Besides, it may be considered as an indication of the fact that the hepatic phospholipids are being readily broken down and the components derived thereof used for the synthesis of neutral lipids. On the same basis, the slight increase in phospholipid content of the liver noted on the 5th day, in the light of a corresponding fall in total lipids and glycerides (chapter - 4, section II) could also be easily explained. From the Table - 1, Figure -1, it becomes clear that the hepatic cholesterol content shows an opposite pattern of changes in the form of an increase on the 3rd day and a decrease on the 5th day. The corresponding fall in blood cholesterol level observed on the 3rd day appears to indicate a possible mobilisation of cholesterol by liver from the blood. However, during 3rd and 5th days, both

the hepatic as well as blood cholesterol fractions were seen to undergo a fall, denoting both, a simultaneous release from the liver, as well as, an active withdrawal from the blood. Possible uptake of cholesterol by the healing tail tissues at this period for meeting the requirements of the developing granulation tissue also may be expected. Even the participation of cholesterol in the transport mechanism of unsaturated fatty acids too, cannot be ignored. Moreover, the slight increase in total blood lipid value obtained on the 3rd day postamputation (chapter - 4, section II) appears to be mainly due to the phospholipid fraction, as hepatic cholesterol, glycerides as well as phospholipids were all noted to show an upward trend on the 3rd day, with the blood cholesterol level remaining low.

The most striking feature of the present study is the continued steady depletion of phospholipids from the liver even on the 7th and 12th days postamputation, which usually correspond with the completion of wound healing in Calotes. The levels as they stand on the 7th and 12th days are representative of approximately 65 and 85% depletion

respectively from the normal value. The fall in phospholipid content from the liver can be explained on the basis of its mobilisation to the wound site, as, an increase in the phospholipid with the growth in granulation tissue subsequent to wound healing has been reported in mammals. Some pertinent reports in this connection are those of Bole et al. (1962), who showed that in guinea pig, with the progressive formation of granulation tissue, the phospholipid content too increased until the 21st day when it proved to be as high as 50% of the total lipids, consisting of such typical phospholipids as phosphatidyl ethanolamine and serine (cephalin), and lesser amounts of lecithin, lysolecithin and spingomyelin; Levin and Head, (1965), who showed that from 3rd to 8th day, the phospholipid content in the healing wound increased from 46.4% to 58.8%; and, Jackson (1964), who showed, that, in cutaneous wounds the phospholipid content increased from 29% at day three to 58% at day eight. Viewed in this background, a fall within reasonable limits of the hepatic phospholipid content is though understandable, the precarious fall noted herein is, however,

untenable and hence merits other considerations. The most relevant in this connection is the concomitant infiltration of neutral lipids and the histometamorphosis of the hepatic tissue into a fatty liver observed on these very same periods (chapter - 4, section II). It may be pertinent in this context to conjure up a sort of temporal and spatial molecular interrelationship between phospholipids and neutral lipids. If this conjecture be accepted, then it is quite possible that the phospholipolytic products are being channelised into the synthetic machinery of neutral lipids. However, why such an interchange occurs, would, still remain obscure. Nevertheless, the tremendous increase in the cholesterol content noted on the 7th day is rather interesting and might possibly offer some clue to the understanding of the changes that are in operation in the hepatic tissue at this period. This high level of cholesterol taken together with only a little change observed on the 12th day may be suggestive of the formation cholesterol esters. Since it is known that cholesterol competes actively with phospholipids for unsaturated fatty acids, an increased content of cholesterol might be very effective in robbing most

of the available unsaturated acids, otherwise available for phospholipid formation, leading to an almost negative phospholipid turnover. Such an increase in cholesterol and cholesterol esters has been associated with fatty livers of differing etiology (Popper and Schaffner, 1957). In fact cholesterol has been by itself considered as a direct potent stimulus for fat infiltration (Peters and Van Slyke, 1946). Another point which might further accentuate this problem is the slow rate of release of cholesterol esters to the blood (Popper and Schaffner, 1957) which corroborates well with the presently noted very little change in the cholesterol content of liver as well as blood on the 12th day (Table - 1, Figure - 1). In this quizical pattern of hepatic lipid metabolism during postamputation of tail, even if cholesterol be considered as a motivator of pivotal significance, the possible source of this sterol still remains enigmatic and eludes a precise explanation. It may be appropriate in this wake to ponder as to whether there is a mobilisation from other peripheral organs, stepped up synthesis within the liver or an increased uptake and conservation from the diet.

Subsequently on the 25th day there is a considerable increase in the phospholipid content of the liver which corresponds with the decreasing total lipid and glyceride contents and a marked recoupment in the histological feature of the liver (chapter - 4, section II). The data on the liver cholesterol which again shows a further fall, might be closely related, and could be indicative of the continuing release of cholesterol esters. The paradoxical increase (though not very significant), in the cholesterol content of blood from the 5th day onwards, and the attainment of a very slightly above normal value on the 25th day also could be taken in support of this contention. The increase in the phospholipid content in the liver may be attributed to the increased lipolysis and a rerouting of the glyceride moieties towards phospholipogenesis. Incidentally, the controlling mechanism which has brought about such a dramatic reversal of the intricate course of events that had occurred in the previous period, is once again a matter of conjecture and precludes any reasonable explanation at this juncture.

Finally, an introspection of the various facts

of hepatic lipid metabolism that have been reviewed in these two chapters appears to give a definite indication, that the regenerative process elicit^{as} a different pattern of hepatic response in the animal with a disposition towards regeneration, in contradistinction to the one with no such potential.