CHAPTER - 7

SECTION: I EFFECT OF TAIL REGENERATION ON THE TOTAL HEPATIC NUCLEIC ACIDS, PROTEIN AND WATER CONTENTS IN THE SCINCID LIZARD, MABUYA CARINATA

Since regeneration essentially involves developmental events such as cell division, differentiation and growth, quantitative as well as qualitative changes in nucleic acids and proteins could be obviously expected to take place at the local site. Such changes have in fact been demonstrated in both amphibian limb and tail regeneration (Yakoleva, 1943a, 1943b; Clement-Noel, 1944; Roskin and Karlova, 1944; Litwiller, 1939; Hay and Fischman, 1961; Osteen and Walker, 1961; Riddiford, 1960; Orekhovich and Sokolova, 1940; Deuchar et al., 1957; Bodemer and Everett, 1959; Anton, 1961, 1965; Quinn, 1962; Schmidt and Woerthwein, 1966; Schmidt, 1966d, 1966e, 1968), and reptilian tail regeneration (Shah and Chakko, 1967, 1969; Radhakrishnan, 1973). All these studies were focused on the site of regeneration itself, and have enabled in establishing the fact, that during regeneration, nucleic acids and protein do show phase specific changes. Since nucleic acids and protein are important in many biological processes, and as significant changes in these constituents have already been demonstrated at the local site of regeneration itself, the possible involvement of hepatic tissue vis a vis nucleic acid and protein metabolism during regeneration, would be a worthwhile exercise, as liver represents the central biochemical machinery of a vertebrate body. This would also enable in elucidating another aspect of the systemic response in the regenerative mechanics. Moreover, in the light of the observed metabolic changes involving glycogen and lipids (Section - 1, Chapters- 3, 4, 5), and even haemopoietic changes (Section - 1, Chapter-2), an investigation involving the changes in the nucleic acid and protein contents of the liver during regeneration would be profitable in clearing the overall picture of the metabolic interrelationships in operation during tail regeneration in the scincid lizard, Mabuya carinata. It was in this background, that a quantitative evalution of the total content of DNA, RNA Protein and water in the liver of the lizard, during different phases of tail regeneration, was undertaken.

MATERIAL AND METHODS

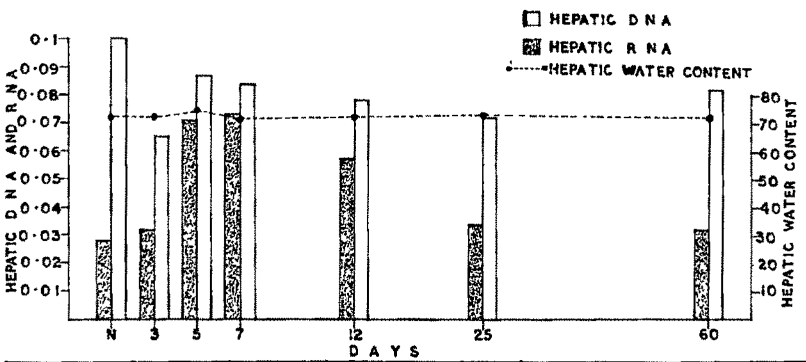
Adult Mabuyas obtained from Karnataka, India, and maintained on a diet of insects in the laboratory were used as the experimental animals for the study. The animals were allowed to get acclimatized to the laboratory conditions prior to tail autotomy. Subsequently on the specified periods after tail autotomy (at different stages of tail regeneration), the animals were sacrificed and liver samples collected for the quantitative analysis of nucleic acids, protein and water. The estimations of DNA, RNA, protein and water content were done as per the methods of Schneider, (1957) and Lowry et al., (1951) respectively.

RESULTS

NUCLEIC ACIDS

The changes in the nucleic acid content (DNA & RNA) are represented in Table - 1, Figure - 1. The most significant change was that of RNA which showed a gradual increase subsequent to autotomy and attained a maximal level on the 5th day which was maintained so even on the 7th day. Thereafter, the

FIG 1: TABLE·I CHANGES IN THE HEPATIC DNA RNA AND WATER CONTENT IN MABUYA CARINATA



TABLE·I	NORMAL	3RD DAY	5TH DAY	7TH DAY	12TH DAY	25TH DAY	60TH DAY
HEPATIC DNA	0·106± 0·005	0·065± 0·012	0·087± 0·012	0·084± 0·015	0·078± 0·028	0 072± 0·023	0·082± 0·011
HEPATIC RNA	0·028± 0·009	0·032± 0·015	0·071± 0·009	0·073± 0·015	0·057± 0·011	0·034± 0·006	0·032± 0·011
HEPATIC WATER CONTENT	72·85± 2·78	72·10± 2·34	74·97± 2·54	71·86± 2·50	72·17± 3·57	73 57± 3·76	72·60± 2·83

FIG·2: TABLE·2· CHANGES IN THE RNA/DNA RATIO AND PROTEIN IN MABUYA CARINATA

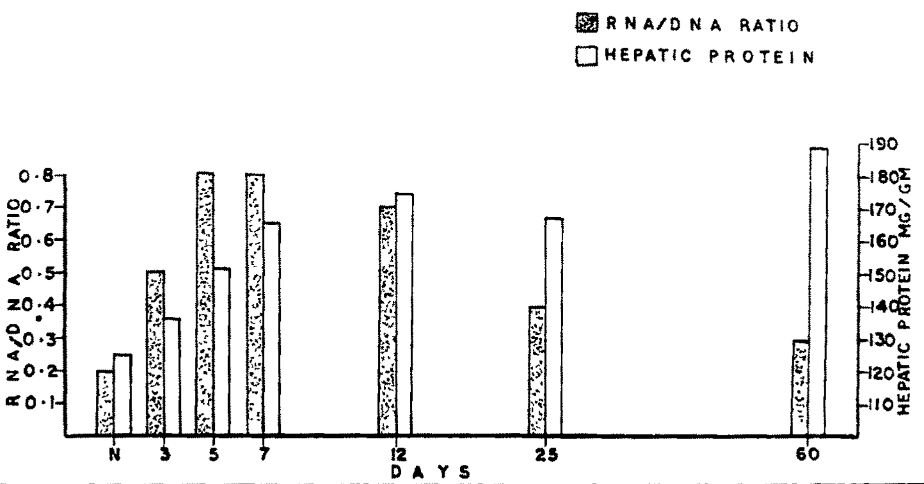


TABLE.2	NORMAL	3RD DAY	5TH DAY	7TH DAY	12TH DAY	25TH DAY	60TH DAY
HEPATIC RNA/DNA.RATIO	0·20	0·50	0·80	0·80	0·70	0·40	0·30
HEPATIC PROTEIN	125·1 ± 9·64	136·0 ± 4·21	151·0 ± 12·04	165·1 ± 6·06	174·9 ± 7·98	167·9 ± 13·85	189·0 ± 5·59

RNA content though showed a gradual decline, neverthless maintained a slightly above normal level on the 25th as well as the 60th days of tail regeneration. In comparison, the DNA content showed a slightly reduced level all throughout the period of regeneration with a pronounced fall on the 3rd day.

PROTEIN

The protein content of the liver was seen to show gradual and continuous increase right from the time of autotomy uptil the 12th day. But there was a fall in its level after the 12th day which became quite abvious on the 25th day. On day 60, the hepatic protein content was noted to be at a maximal level (Table - 1, Figure - 1).

WATER CONTENT

The hepatic water content appeared to remain more or less unchanged all throughout regeneration excepting for a slight increase on the 5th day.

DISCUSSION

In the previous chapters (3, 4, 5), the involvement of hepatic lipids and glycogen in the regenerative process as a part of the systemic response has been pointed out. The changes observed in the present study on the hepatic nucleic acid and protein contents too, tend to emphasize the greater involvement of liver in meeting the exigencies of tail regeneration in the scincid lizard, Mabuya carinata. It becomes apparent from the present study that alongwith lipids and glycogen, hepatic protein too shows a high turn over during regeneration. The protein content was seen to register a continuous increase through 3rd, 5th and 7th days postautotomy and attained a high level on the 12th day. The corresponding increase observed in the case of RNA on the 3rd day and the maintenance of its higher level on the 5th, 7th and 12th days are in good correlation with the increase in protein. Moreover, the RNA/DNA ratio, which is usually indicative of protein synthesis, was also seen to register a continuous increase ultimately reaching a plateau on the 5th and 7th days of regeneration. After the 7th day, the ratio began to decline gradually which became quite obvious on day 25th. All these are indicative of a high protein turnover in the liver during the early phases of regeneration. The increase in both RNA as well as protein contents observed during the early periods of regeneration could be attributed to on the one hand, the increased lymphocytopoietic activity (chapter-2), and on the other hand to the increased metabolic activity as marked by the enhanced activities of the enzymes involved in intermediary metabolism (Unpublished data). Moreover, the influx of R.B.Cs and their destruction, as well as the increased haemoglobin content of the blood too (chapters- 1, 2; both involving globin) could also be the contributing factors in the increased content of hepatic proteins. The fall in RNA content as well as protein content between the 12th and 25th days of regeneration (Table - 1, Figure - 1) are well paralleled by the declining activities of the enzymes of intermediary metabolism (Unpublished data). Besides, the fall in the protein content could be indicative of the increased mobilization of proteins from the liver, especially to the site of regeneration.

Changes in the total nitrogen content of the regenerate as well as the subjecent stump tissues in amphibians have been reported by Deucher _et al._, (1957) as well as Schmidt (1968). Though there are slight differences in the two reports, both the groups of workers have indicated a definite decline in the total nitrogen value of the appendage during the early periods of regeneration after amputation. In this light, the possible regurgitation into the blood stream of the protein breakdown moities, produced at the wound site after autotomy, and the resultant accumulation in the hepatic tissue cannot be overlooked. It is also pertinent to note, at this juncture, that the hepatic protein content of rats treated with cortisone was seen to undergo an increase (Goodland and Munro, 1959). Such an increase in liver protein content has been suggested to be due to mobilization from other sources especially muscle under the influence of cortisone (Munro, 1964). In the wake of the known fact that cortisone lowers the tensile strength of the wound by reduced collagen fibrillogenesis and connective tissue formation (Sandberg, 1963b; 1964b); and this being a prerequisite for regeneration, the involvement of cortisone in the wound healing mechanics associated with regeneration has been hinted at by Goss (1969).

In this light the present increase in hepatic protein content noted during the early periods of regeneration appears self explanatory and could be considered in part to be a cortisone mediated one. Another interesting observation in this connection is that of a stress induced (including surgical stress) increase in the hepatic protein content mainly involving plasma proteins in rats (Majumdar et al., 1967). Thus the currently noted continuous but gradual increase of hepatic protein content during tail regeneration in the first twelve days after autotomy in Mabuya carinata could be attributed to the probable involvement of all the factors mentioned above in varying degrees.

Though a regular supply of protein moieties or their components from the liver to the regenerating system all throughout could be easily visualised, the noticeable fall in the protein content of the liver recorded between the 12th and 25th days postautotomy is rather striking and might highlight the depletion of protein from the liver to meet the increased exigensies of the regenerate, as this period corresponds with the commencement of histodifferentiation.

Interestingly, Schmidt (1966c) based upon the study of seperation of soluble proteins of regenerating tissues on polyacrylamide gels, identified the appearance of 3 new bands during the early period of regeneration, and 4 additional bands in the critical period of transition from morphological indifference of the blastema cell to the initial histogenesis of differentiation phase. Though some of these additional bands could be attributed to the appearance of the many enzymes characteristic of regeneration, some of them could also be regarded as "regeneration promoters" as hinted at by Schmidt (1968). Again Deck and Goldman (1963) and Deck (1965) had succeeded in initiating the formation of regeneration blastema in normally nonregenerating, denervated, adult newt limbs by infusing the extracts of regenerating tissue or nerve tissue. However, none of the blastemas differentiated into a recognizable limb suggesting the need for some other inductive mechanism. It could be safely assumed from these that the operation of phase specific protein promoters or inductors are in order, and, when viewed in the backdrop of the present investigation on the hepatic protein content is rather tempting, and, the possible participation of the hepatic tissue in the elaboration of these proteins either in their specific form itself or even in the unfinished nonspecific form (which could be then modelled according to the specificities within the regenerate itself) could be tentatively assumed. Further evidences in favour of such an elaboration by the liver comes from the increase in the RNA content as well as the RNA/DNA ratio obtained in the current study during the early periods of tail regeneration (Figure - 2, Table - 2).

With the progression of differentiation and the attainment of an advanced condition of the regenerate, the burden of elaborating new protein molecules also appears to come down as could be made out from the fall in the both the RNA content as well as the RNA/DNA ratio of the liver in Mabuya carinata during its tail regeneration from the 12th day postautotomy onwards. However, the high level of protein content observed even on day 60 of regeneration needs a passing reference and may have to be viewed in the background of the earlier observed massive influx of R.B.Cs and their destruction within the liver about the 25th day of tail regeneration onwards. In this respect the higher levels of the hepatic protein content noted towards the end of regeneration may have to be attributed to such extrahepatic sources including diet, and even a general anabolic influence (Chapter - 6) rather than an intrahepatic synthesis. The possible contribution by the degenerating blood cells is further emphasized by the observed increase in the iron content of the liver during the later phases of regeneration (Chapter - 2).

The water content of the liver during the various periods of regeneration have not revealed any significant change whatsoever excepting for a slight increase on about the 5th day of the tail regeneration which could be attributed to the geared up metabolic and biochemical activities reviewed and discussed in the present thesis. Similarly, the slight fall in the hepatic DNA content noted during regeneration (Table - 1, Figure - 1) could be perhaps due to the regenerative stress induced hypertrophy of the hepatocytes.

SECTION: II EFFECT OF TAIL WOUND HEALING ON THE TOTAL HEPATIC NUCLEIC ACIDS, PROTEIN AND WATER CONTENTS IN THE AGAMID LIZARD, CALOTES VERSICOLOR

Though a number of workers have investigated the changes in the content of nucleic acids and proteins during wound healing in mammals (Tsanev, 1963; Williamson, and Guschlbauer, 1963; Guschlbauer and Williamson, 1963; Forscher and Cecil, 1957; Scheving and Chiabulas, 1965; Bashy *et al*., 1964; Beknor and Bavetta, 1965; Jackson *et al*., 1964; Udenfriend, 1966; Reynolds *et al*.,1963), the involvement of visceral factors have received no attention at all, even amongst the mammals. In the previous chapters, the involvement of hepatic glycogen and lipids in the tail wound healing mechanics in *Calotes versicolor* has been clearly brought out. In this light, the changes undergone by the hepatic protein as well as nucleic acid contents would enable in establishing not only the involvement of hepatic protein as a part of the systemic response in the normal wound healing mechanics in lizards but also would help in understanding the overall picture regarding the metabolic intricacies of the liver in the wound healing mechanics of <u>Calotes versicolor</u>. Moreover, in the previous section the hepatic preteins were seen to show an increase during tail regeneration in Mabuya, which emphasized the quantitative requirement of proteins in regeneration, as well as the importance of the liver in meeting the requirements. It becomes imperative in this connection to study the changes in the hepatic protein content as well as nucleic acid contents during tail wound healing in the Agamid lizard, <u>Calotes versicolor</u> where regeneration does not occur, so that not only the differences if any involved between regeneration and wound healing in the lizards could be made out, but also could serve as a suitable control to the studies on regeneration.

MATERIAL AND METHODS

Adult Calotes obtained from the local animal dealer and maintained on a diet of insects were used as the experimental animals. The animals were allowed to get acclimatised to the laboratory conditions for about a fortnight prior to the experimentation. The tails were then amputated at a fixed distance from the vent, and the liver samples from normal (with intact tail) as well as animals with healing tail wounds were obtained by sacrifising the animals under mild anaesthesia at regular intervals. The quantitative evaluation of nucleic acids (DNA & RNA), proteins, and water content in the liver was carried out as per the methods of Schneider, (1957); Lowry et. al., (1951).

RESULTS

The changes undergone by the three parameters studied herein are depicted in Tables- 1, 2; Figures- 1, 2. The changes in the water content appears to be more or less reciprocal to the changes in the lipid content of the liver (Section - II, Chapter - 4).

NUCLEIC ACIDS

Unlike in the case of Mabuya, as noted in the previous section, in Calotes, the changes in the nucleic acid contents of the liver appeared to be very insignificant. The DNA content showed a slight increase on the 5th and 7th days which by day 25 became very

FIG. I TABLE. I. CHANGES IN THE HEPATIC D N A R N A AND WATER CONTENT IN CALOTES VERSICOLOR

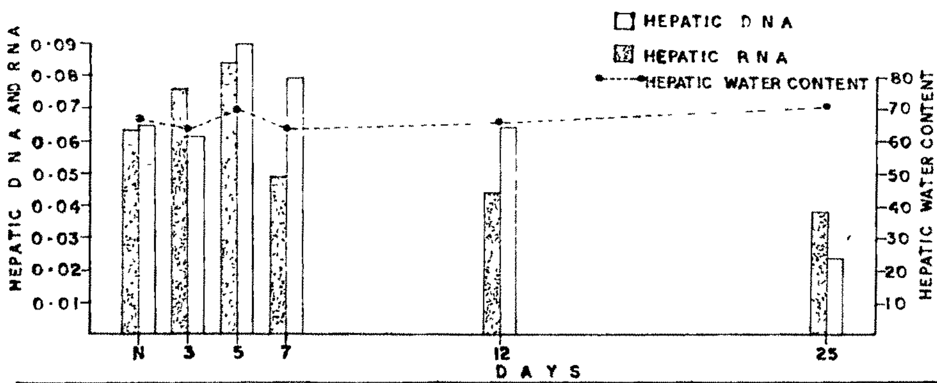


TABLE · I	NORMAL	3RD DAY	5TH DAY	7TH DAY	12TH DAY	25TH DAY
HEPATIC D N A	0·064± 0·023	0·061± 0·021	0·090± 0·019	0·079± 0·020	0 064± 0·013	0·024± 0·010
HEPATIC R N A	0·063± 0·016	0·076± 0·032	0·084± 0·027	0 049± 0·010	0·094± 0·017	0 038± 0·013
HEPATIC WATER CONTENT	66·41± 3·84	63·95± 6·85	69·27± 6·04	63·82± 7·54	65·09± 3·38	71·92± 5·59

FIG-2: TABLE-2: CHANGES IN THE RNA/DNA RATIO AND PROTEIN IN CALOTES VERSICOLOR

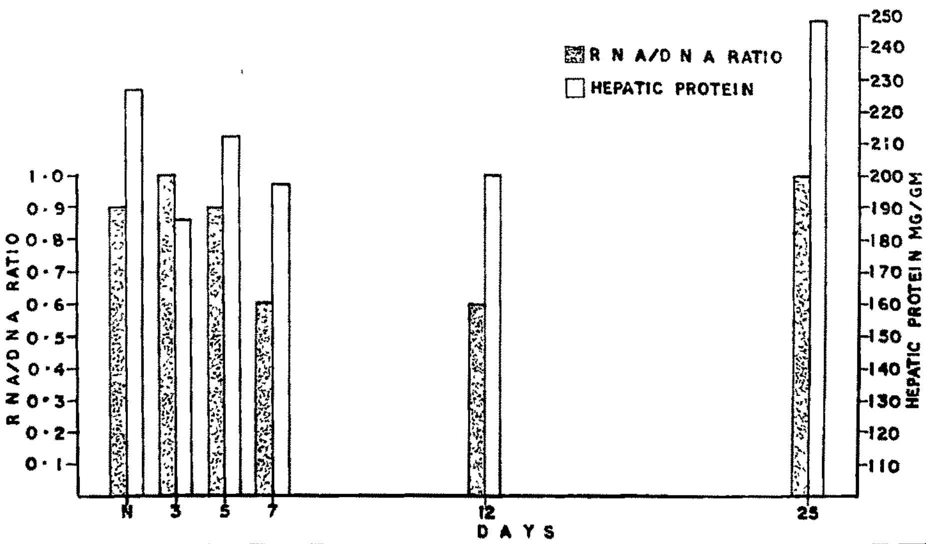


TABLE-2	NORMAL	3RD DAY	5TH DAY	7TH DAY	12TH DAY	25TH DAY
HEPATIC R N A/D N A RATIO	0·9	1·0	0·9	0·6	0·6	1·0
HEPATIC PROTEIN	226·1 ± 36·6	186·9 ± 36·6	212·2 ± 34·3	197·3 ± 44·2	200·3 ± 26·8	248·9 ± 41·8

much subnormal. On the other hand the RNA content showed a slight increase on the 7th, 12th and 25th days. The RNA/DNA ratio remained unchanged excepting during the 7th and 12th days whence its value showed a fall, and, on days 3 and 25, there was a tendency for the value to be slightly more than the normal.

PROTEIN CONTENT

The hepatic protein content registered a significant fall on the 3rd day postamputation to be followed by a slight increase on the 5th day (which however was still subnormal), only to show a further fall on the 7th day. Since then, by day 12, the hepatic protein content increased and ultimately on the 25th day the protein content reached an above normal level.

DISCUSSION

Investigations carried out on the injury (of various types) induced metabolic response in mammals have established severe to moderate loss of body proteins and the setting in of negative nitrogen balance in the animals (Cuthbertson, 1936;

Cuthbertson, 1964). However, extensive investigations carried out by a number of workers (Cuthbertson et al., 1939; Munro and Chalmers, 1945; Fleck and Munro, 1963; Munro and Cuthbertson, 1943; Callowy et al., 1955; Madden and Caly, 1945; Browne, 1944) have brought out a differential catabolic response to injury based on differences in the dietary factors as well as on the nutritional state of the animals. Further studies by Munro (1964), and Munro and Waterlow (1965) and that of Fleck and Munro (1963) led to the conclusion that animals with a higher protein intake had a greater protein content in the liver (labile protein), and subsequently show marked protein depletion after injury, as compared to the animals on a lesser protein intake which had lesser hepatic protein content and show very little protein loss. In this light, the differential response of the hepatic protein content noted during the early periods after tail autotomy and amputation in the case of Mabuya and Calotes respectively, appears to be quite interesting. The significant depletion of the hepatic protein content recorded in the case of Calotes (Table - 2, Figure-2) as compared to the increase observed in the case of Mabuya could be suggestive of the loss of labile proteins as a general catabolic response to injury. Interestingly enough, the protein content of the liver in the case of Calotes is nearly double the amount in the case of Mabuya and thus might represent a large pool of labile proteins in Calotes. However, the diametrically opposite pattern of change observed in the two cases might also signify the differences involved in the two healing mechanics.

Though the slight increase in the RNA/DNA ratio obtained on the 3rd day postamputation could be explained in terms of the increased incidence of various enzymes (Unpublished data) as well as the increased content of protein noted on the 5th day, the gradual increase in the content of both the nucleic acids during the 3rd and 5th days, in the wake of the decreased protein content on the 5th day, are rather intriguing and may have to be viewed in the light of the possible metabolic intricacies that may be associated with the development of fatty liver (Section - II of Chapters - 2 & 4). However, the subnormal levels of hepatic protein recorded on the 7th and 12th days might be indicative of the possible supply of protein moieties to the healing wound of the tail and/or the possible interconversion of protein components into lipids associated with the enhanced hepatic steatogenesis observed at this period. Moreover, the fall in the content of nucleic acids, (Table - 1, Figure - 1) as well as the RNA/DNA ratio on these days are also suggestive of a repressed protein turnover. At the same time the increase in the cell volume resulting due to the deposition of lipids and the development of fatty liver might also be responsible for the noted decreased values of the nucleic acids on the 7th and 12th days postamputation.

Subsequently, though the increased RNA/DNA ratio as well as the above normal hepatic protein content observed on the 25th day may be correlated with the reversal and recovery of the liver from steatogenesis and fatty liver condition (Section - II, Chapters-2&3), the concomitant subnormal levels of DNA and RNA recorded on the 25th day remain somewhat enigmatic. Presumably it may be tentatively surmised, that in the wake of the development of fatty liver and its subsequent recovery, some of the hepatic tissue may undergo disintigration thus leading to a fall in the total DNA and RNA content of the organ on the 25th day. Possible alternative explanations as well as detailed understanding of the whole problem would require further investigations directed on these lines.

Finally, the changes in the water content of the hepatic tissue during the various periods of tail healing have shown a decrease during the earlier periods and an increase during the later periods (Table - 2, Figure - 2) which correspond inversely with that of lipids (Section - II, Chapter - 3).