

INTRODUCTION

Regeneration a fascinating biological phenomenon which represents the replacement of lost parts of an organism is fast gaining wider attention and forms an important, if not the main theme of study in the field of developmental biology. It serves as a very novel system for understanding the principles and mechanics of development and differentiation. As this process of replacement of lost parts forms a miniature world of embryonic development within an otherwise normal fully developed and differentiated animal body, it minimises many of the difficulties and problems involved in the study of ontogenesis. The regenerating system as it simulates many of the events of normal development, serves as an excellent medium for understanding the modus operandi of various developmental aspects involved in embryogenesis. Another interesting point is the fact that contrary to normal embryonic development, the regenerating system starts and completes its development in an environment of fully differentiated and mature tissues. In this respect, the regenerating system cannot be studied in total exclusion of the adult parental body to which it is

attached and which forms an integral part of its total structure and function. This difference in the environmental molecular ecology is the most striking feature of regeneration in comparison to that of normal development. Whereas the controlling factors are inherent within the developing egg itself during ontogenesis, in the case of regeneration these must necessarily originate secondarily from within the animal body. Are these factors inherent at the local site itself, or are they invoked from distant parts of the body? If so, how, when and where are they formed, and, how do they act, so as to not only initiate, but also maintain the progress of regeneration by supplying the necessary impetus? And once elicited and initiated, the regenerative process would go to completion uninterrupted, or, are they required to be present continuously? And last but not the least, the factors present and/or produced by the two different ecological niches i.e. regeneration and ontogeny, are similar or dissimilar? These are the queries which plague the field of developmental physiology and answers to which when found would go a long way in solving

many of the mysteries and intricacies surrounding the process of development and differentiation. Though the queries appear rather simple and easy to solve, they are really far fetched and uncertain, and cannot as yet be answered. It is in this context that the regenerating system serves as an excellent medium for investigating the varied aspects of developmental biology. However, a beginning in this direction could be made by attempting to study the physiology of the animal as a whole in relation to regeneration of its lost parts.

Much of the earlier investigations on regeneration have been focused on the regenerating fins and limbs of fishes and amphibians (Goss, 1969; Gekzik and Wolsky, 1959; Niwelinski, 1960; Schmidt, 1966; Wolf and Cohen, 1963). Investigations of this sort were lacking in the case of reptiles and, to fill in this gap, studies on histomorphology, histophysiology, histoenzymology and biochemistry of the regenerating tail had been undertaken in this laboratory on two lizards, Hemidactylus flaviviridies and Mabuva carinata.

These studies clearly brought out the existence of certain interesting metabolic and biochemical changes during tail regeneration in lizards. All the studies referred to above involving, both, the reptiles as well as amphibians and fishes have, however, been directed to the site of regeneration itself i.e. to the respective regenerating appendages. In this context, an attempt to understand the possible changes in the physiology of the animal in relation to regeneration would be a worthwhile exercise, and would throw more light on the phenomenon of regeneration. Moreover, as the regenerating system remains firmly attached and associated to the parent animal body, it becomes all the more pertinent, and would help us in not only correlating the changes observed in the regenerating appendages with the physiology of the animal but also possibly help in elucidating the probable systemic factors controlling the process itself. Some such interdependency between the regenerating system and the parental body being a definite certainty, the present study is a sequel to this line of thinking and involves an

aspect totally overlooked and unattended to. This possibly might represent the fundamental difference between ontogeny and regeneration. It is with this intention that studies on the histophysiology of certain organs such as liver, spleen, bone marrow, kidney, fat bodies, and blood, during tail regeneration in Mabuya carinata were undertaken. Simultaneously, studies have also been conducted on Calotes versicolor during wound healing after amputation of its tail, so as not only to understand the physiology of the animal in relation to wound healing in reptiles in general, but also to help serve as controls for the studies on Mabuya, since tail of Calotes does not regenerate once amputated. Moreover, such comparative study would also enable us to understand the possible differences if any between the two types of wound healing, one that occurs prior to regeneration and the other where no regenerative event follows. To avoid the contradictions and errors that might arise due to seasonal changes if any, the present study was carried out within a short span of three months viz. during the period of January-March.

In order to evaluate the possible as well as extent of involvement of blood in the process of regeneration, an analysis of blood in terms of its cellular composition and haemoglobin content has been undertaken. The investigation proved rather interesting and showed definite changes. These changes have been construed to indicate the involvement of haemopoietic system in the mechanics of regeneration and the various changes that are observed in the blood are discussed in the text in terms of metabolic intricacies at the site of regeneration, cellular source for the formation of regeneration blastema and role of monocytes during regeneration respectively. Calotes on the other hand failed to show any such spectacular changes and whatever little changes were evidenced are correlated with the normal wound healing process in a reptile.

In response to the above observed changes in blood during regeneration, a detailed histophysiological study of the haemopoietic organs such as liver, spleen and bone marrow was undertaken. During regeneration, all these organs are found to undergo histophysiological changes. Increased haemopoietic

activity in the marrow and hypertrophy of the white pulp are the important changes shown by bone marrow and spleen respectively. In the liver the most marked effect appears to be the formation of lymphocytopoietic nodules. Another interesting observation is the influx and destruction of R.B.Cs both in the liver and spleen; once during the early regressive phase and once during the late differentiation (By about 25th day) phase of regeneration. An attempt is made to correlate these changes and observations in the three haemopoietic organs with possible involvement of lymphocytes in the formation of a regeneration blastema and also a possibility of haemoglobin transition during regeneration. Again similar studies carried out on Calotes failed to show changes as were seen in Mabuya and thus confirmed the fact that the regenerative process initiates a number of specific events in contrast to the normal nonregenerating system where only wound healing occurs.

Earlier investigations from this laboratory on tail regeneration in Mabuya (Shah and Ramachandran,

1970, 1972, 1973, 1974, 1975; Ramachandran et al., 1975; Radhakrishnan and Shah, 1973; Radhakrishnan, 1972) had established certain distinct changes with regard to the pattern of metabolism of the regenerating tail. It was suggested by these workers that there is a metabolic flux during regeneration. Liver being the central organ for all biochemical as well as metabolic changes, it was deemed fit to investigate quantitatively the level of metabolites like glycogen, total lipids, phospholipids, cholesterol, and protein and also the level of ascorbic acid, nucleic acids and percentage of water content, so as to understand the extent and mode of involvement of liver in the regenerative process. To make the study better understood, the levels of glucose, lipids and cholesterol in the blood too, were assayed.

Changes in the hepatic glycogen content and blood glucose showed a more or less mirror image pattern. Hepatic glycogen content was found to decrease during the wound healing as well as early differentiation phases. Concomitantly the blood glucose level was found to remain at an elevated

level during these two periods. These changes are construed to indicate the utility value of hepatic glycogen and blood glucose during these phases of regeneration. A fuller review on this aspect in relation to the changes observed in the tail is taken up in the text. The data on Calotes appear to give a less significant change. As such, it could be easily surmised that a mere wound healing in the tail in lizards does not influence much, the hepatic glycogen and blood glucose concentrations; whereas the regenerating process definitely invokes certain intricate and more dramatic changes in these two metabolites.

Lipids were found to play a very significant role in the molecular ecology of blastema and differentiation phases (Shah and Ramachandran, 1970, 1973, 1974, 1975; Radhakrishnan, 1972). Since lipids serve as the chief reserve energy substrate and as liver is known to function as an important centre for storage as well as metabolism of lipids, the regenerative phenomenon with its tremendous metabolic needs could be supposed to invoke certain specific or generalised response. In this light a

quantitative evaluation of the total lipids, glycerides, phospholipids and cholesterol was carried out in the liver during tail regeneration. To have a better understanding in this respect, the level of total lipids as well as cholesterol in blood were also estimated. Though the changes in the hepatic lipid content were not as dramatic as in the case of glycogen, there was nevertheless noticeable changes which are being correlated not only with the biochemical necessities of the process of regeneration itself but also with the interdependency of intermediary metabolism and the necessities of the various other systems working in unison to support the process of regeneration. However, changes in the blood lipid content are more dramatic and remained low all throughout the period of regeneration with no signs of recovery to normal level even as late as the 60th day of regeneration. This appears to have relevance in the context to the changes observed in the regenerating system, liver, visceral fat body, haemopoietic system etc. Similar studies on Calotes yielded divergent pattern of changes which highlight

the differential response of the body in the regenerative mechanics in comparison to normal wound healing. The slight increase in phospholipid content noted during the early periods of regeneration is correlated with the haemopoietic involvement of the organ. Cholesterol was, however, seen to show fluctuations, and an attempt is made to correlate these fluctuations with the differential synthesis and secretion of various cholesterol based hormones which might be purported to play a significant role during regeneration.

Another interesting aspect thought worthwhile to investigate was the role of visceral fat bodies in the regenerative mechanics. The two parameters studied in this respect were the percentage weight of the fat bodies in relation to body weight and the histological appearance during different phases of tail regeneration. Both these parameters gave definite indications of a depletion of lipids from the fat bodies during the regressive phases of regeneration. An interesting and significant outcome of the histological investigations was the haemopoietic nature of the fat bodies in Mabuya.

Stimulated erythropoietic activity could be visualised during early periods of regeneration and is considered to be functioning as a satellite organ in this respect. None of these changes or observations could be visualised in the fat bodies of Calotes during its tail wound healing after amputation. Hence it becomes amply clear that the fat of the fat bodies in Calotes does not play any role in the normal wound healing process.

To have a better understanding of the hepatic response in regeneration as well as wound healing, the percentage of water content, protein, DNA and RNA in the organ were also estimated. These values also underscore a greater involvement of the systemic factors during regeneration than in wound healing. The changes in the protein content were noted to be divergent in the two lizards, with a more pronounced effect in Mabuya. It was seen that the hepatic protein content undergoes an increase in response to the regenerative process whereas during mere normal wound healing in Calotes there was a slight decrease. This could be attributed to the increased protein necessity of the regenerating

elements and the involvement of the liver (both direct as well as indirect) in meeting this exigency of protein requirement. This is also correlated with the earlier mentioned haemopoietic response of the liver. The nucleic acid contents of the liver were noted to be more or less parallel to that of protein. In Mabuya, the DNA content of liver was seen to show a slight reduction during regeneration. This is probably due to the hypertrophy of the hepatic cells which in fact is so observed histologically. The RNA/DNA ratio was also found to increase during regeneration indicating enhanced protein synthesis. On the other hand, in the case of Calotes, this value never went up above the normal level. The percentage of water content in both the cases was found to show reciprocal changes with the total lipid content.

Ascorbic acid has been shown to play significant role in the regenerating tail of Mabuya (Ramchandran et al., 1975). These authors had shown a two fold as well as a five fold increase during wound healing and differentiation phases of

regeneration respectively. To have a better understanding of the role as well as source and supply of ascorbic acid, quantitative estimations of this vitamin was carried out in liver and kidney of Mabuya. Such an investigation demonstrated the greater involvement of kidney in comparison to that of liver. Similar studies carried out on Calotes also demonstrated more significant changes in the ascorbic acid level in the kidney. It could be surmised from this that the renal tissue in these lizards play a principal role as the supplier of ascorbic acid for wound healing as well as regeneration as the case may be. The principal difference between the two cases lies in the fact that whereas Calotes responded by a decreased or depleted content of ascorbic acid during its wound healing process (confirmed by the increasing level of this vitamin in the healing tail), Mabuya responded by an increased content and elevated synthesis of ascorbic acid during its tail regeneration. This could be not only correlated with the increased necessity of the ascorbic acid for the various functional aspects of the

regenerating tissues but also with the synergistic functioning of various other visceral organs.

Finally, as a continuation of the many earlier enzymological investigations carried out in the regenerating tail of Mabuya, three more enzymes viz. ATPase, hexo and glucokinases and glutamate dehydrogenase were investigated in the regenerating tail using histochemical techniques. These investigations demonstrated enhanced activities of all the three enzymes during the various phases of regeneration and have been as such correlated with increased energy flux, glucose utilization as well as protein metabolism respectively. They have also helped in confirming the conclusions reached earlier on the basis of metabolic studies carried out on lacertilian tail regeneration.