

GENERAL CONSIDERATIONS

Histophysiological investigations on regenerating tail, conducted during the last decade in this laboratory (Shah, 1975) have brought to light existence of a well balanced and regulated operation of metabolic pathways underlying the process of tail regeneration in lizards. These investigations have provided us with a stable foundation for pursuing further studies related to systemic and endocrine response to the trauma of loss of an appendage and involvement of these factors in metabolic adjustments aimed at restoration of the lost structures. Organismal adaptive responses, such as repair and regeneration, wherein healing of the wound, formation of blastema, differentiation and growth of the regenerate predominate, are undoubtedly structural manifestations of metabolic adjustments translated into effectiveness through the participation of a number of enzymes, metabolites and hormones. Hence, the process of tail regeneration in lizards as a whole provides an admirable system for elucidating the relationship between metabolic activities of various organs of the body and cellular activities of the regenerate. For last two and a half decades, many biologists have shown immense interest in reptilian tail regeneration. Reptiles are of paramount

interest in the study of reparative regeneration, since as a taxonomic group they fall between amphibians on one hand where regenerative powers are retained to a considerable extent, and on the other hand birds and mammals who have largely lost the ability to regenerate.

Present investigations have been undertaken with a view to understand the metabolic adaptive changes and adjustments occurring in the metabolic pattern of some of the visceral organs during the tail regeneration in the Gekkonid lizard, Hemidactylus flaviviridis. Following considerations are put forth by correlating earlier and present studies in the normal and regenerating tail of this lizard.

Importance of liver owing to its active association with a majority of metabolic events in an animal body need not be overemphasized. The dependency of the regenerating tail on liver for energy purpose could be visualised in the present study. Liver metabolites according to Procaccini (1971) can serve two purposes (i) they can provide a ready source of utilizable energy to escape from the stress resulting from the amputation trauma, (ii) they can also provide precursors for a de novo synthesis of materials necessary for the organ

restoration in question. Shah and Chakko (1967) who noticed depleted levels of glycogen in the tail stump tissue following autotomy through the wound healing phase, suggested glycogen as the chief metabolite used by regenerating tail of the lizard H. flaviviridis during the initial period of the process. The decrease in glycogen content of the hepatic and muscle tissues with a concomitant rise in blood glucose level (Chapter 1) during the wound healing period observed in the present study can be viewed as an index of mobilization of the metabolite for satisfying the energy demands of the broken tail tissues for their repair and healing as well as energy demands of other visceral organs whose activities are geared up at this stage.

Occurrence of wound healing is an essential prerequisite for regeneration and this demands tremendous energy supply. Such a demand is satisfied by active operation of glycolytic and TCA cycle pathways in the liver. It is of interest to note that TCA cycle activity is poor compared to glycolytic pathway reactions at this stage which becomes evident from low activity of SDH and MDH compared to OC-GPDH and LDH in the liver (Chapter 2). However, there was a slight increase in the activity of SDH and MDH compared to their preautotomy levels of

activity. Since hepatic lipid and protein did not change significantly from their respective preautotomy levels during wound healing and blastema stages, it appears that these enzymes (SDH and MDH) act on the substrates derived from glycogen. Thus, overall metabolism during the early period of regeneration is predominantly anaerobic in the liver (Chapter 2) as it is in the tail (Shah and Hiradhar, 1974). This anaerobic mode of metabolism is well reflected in low RBC count and haemoglobin (Hb) percentage recorded during this period (Chapter 4). In this connection, it is interesting to report that lizards in general have low oxygen affinity (Pough, 1969) and as a result they rely heavily on anaerobic metabolism (Bennet, 1972a, b; Bennet and Licht, 1972; Bennet and Dawson, 1972).

Magon (1970), Shah and Ramachandran (1973), Shah and Chakko (1972) and Radhakrishnan (1973) based on circumstantial evidences suggested that glucose moiety in the blastema stage of lacertilian tail regeneration is diverted to HMP pathway leading to production of NADPH_2 , thus aiding in nucleotide and lipid synthesis besides providing energy source for general activities of the blastemal cells. Hence, presently observed reduction in the blood glucose level during blastema stage provides support to above suggestions.

Production of pentose sugars and amino acids is a necessary factor during blastema and differentiation phase, as these products are of utmost importance for the process of cell division and synthesis of proteins. Presently observed increase in the total protein content of the tail tissues during blastema and differentiation phases can be correlated with reported increase in nucleic acid contents in the regenerating tail of lizards (Shah and Chakko, 1972; Radhakrishnan, 1973). Concomitantly observed depletion in the total protein content of the liver during the differentiation phase of the tail regenerate may be for providing amino acids for protein synthesis during the histodifferentiation of the tissues in the regenerate.

The presently observed temporary changes in the metabolic pattern of the liver during different phases of tail regeneration are considered adaptational features to suit the metabolic activities at a required pace, to provide raw materials, cofactors and energy needed for the regenerative process.

From the data on lipid content of the liver during tail regeneration, one is tempted to believe that liver lipids do not partake to a significant extent in the

regenerative process. However, when similar data on adipose tissue is examined, it is realized that there is considerable reduction in its lipid content, when the tail regenerate passes through the differentiation and growth phases. In light of the aforesaid metabolic changes in the liver and adipose tissue during tail regeneration, one is tempted to believe that requirements of the differentiating and growing tissues of the regenerate are satisfied by involvement of lipids from adipose tissue rather than those from liver.

Maintenance of an elevated level of ascorbic acid (AA) in the liver as well as in the kidney throughout the process of tail regeneration is yet another notable feature. The high levels of this vitamin in these visceral organs are indicative of biosynthetic processes occurring there (Chinoy, 1972). Role of AA in the electron transfer mechanism which is an integral part of reactions leading to the energy production may be well accounted for the increased level of this vitamin in these two organs during tail regeneration. Involvement of this vitamin in wound healing has been well established (Gould, 1963). Taking all these facts into consideration, it is logical to visualise that increased mobilization of AA from the liver and kidney to the site of regeneration takes place in order

to meet with the needs that arise for regenerative activities. A quantitative increase in the vitamin content of the regenerate has been reported by Shah et al. (1971) in the lizard, H. flaviviridis. Thus energy requirements for the synthetic and cellular proliferative activities of the regenerate seem to be satisfied by adjustments in availability of metabolites, vitamin and activities of the enzymes in different visceral organs.

In the lizard, H. flaviviridis haemodynamic processes of the body also undergo readjustments following autotomy and during regeneration. Haemopoietic machinery in the spleen and bone marrow gets geared up immediately after autotomy. An augmented lymphocytopoiesis is more apparent during wound healing period and begins to revert back to the normal state once the wound is healed, which suggests that lymphocytes may be well involved directly or indirectly in the healing process (Chapter 4). Similarly a decline in RBC count and haemoglobin content during the wound healing phase could be correlated with a predominantly anaerobic mode of metabolism and reduced oxygen requirements of the animals (Chapter 4), and also with the reports on RQ study made on the regenerating tail of the lizard (Shah and Hiradhar, 1974).

Phosphatases are known to be involved in cell proliferative activities (Olsen and Nordsquit, 1966; Shah and Menon, 1974). It has been noticed that alkaline phosphatase in spleen increases during the wound healing phase when spleen shows hectic cell proliferative activity as inferred from its histological features at this stage (Chapter 5). Thus it is suggested that the activity of this enzyme in spleen is stepped up to adapt to the new situation that has cropped up as a result of autotomy followed by healing and regeneration. As soon as the autotomy wound gets healed and the tail blastema is formed, the augmented lymphocyte production in spleen reduces and concomitantly alkaline phosphatase activity in the organ reaches its preautotomy level. Similarly, increase in the activity of acid phosphatase in the spleen during wound healing and blastema phases of the regenerating tail can be correlated with the increased phagocytic and lytic activities occurring in the organ. These facts imply that changes in the activities of phosphatases in the spleen noticed are adaptive responses to the regenerative process. Observations on splenectomy during regeneration indicated that spleen plays an important role during wound healing phase, and

does not seem to be significantly associated with subsequent phases of regeneration.

In recent years number of developmental biologists have become aware of the fact that hormones play important role in the process of regeneration (Schmidt, 1958a, b; Tassava et al., 1968; Tassava, 1969; Liversage and Scadding, 1969; Turner and Tipton, 1971; Turner, 1972). It has been observed in the present study that the rate of regeneration is greater in male lizards than that in the females (Chapter 6). That sexual difference exerts an influence on the rate of regeneration has been amply demonstrated by Joseph and Dyson (1965) in rabbits and Congdon et al. (1974) in Coleonyx lizards. Such a difference observed in the present study can be attributed to a high level of androgen in the male lizards. However, to prove the anabolic action of androgen on the regenerative process of the lizards, H. flaviviridis, testosterone was administered in males as well as females. And it was interesting to note that testosterone administration accelerated the process of regeneration in female lizards to a considerable extent (Chapter 7). However, testosterone administration did not elicit such a pronounced effect on rate of regeneration in male lizards as was in the females.

Barbera et al. (1962) and Joseph and Dyson (1966) have reported that androgen administration accelerates the healing and regenerative process in mammals. In this connection it is interesting to recall the concept of an "optimum level of androgens" needed for regeneration in mammals (Dyson and Joseph, 1968). It is believed that such could be the condition in the male lizards.

Involvement of thyroid hormone in the regenerative process in these lizards has been examined by removal of the gland, where thyroidectomy caused relatively a greater delay in the rate of growth of the regenerate in the male lizards compared to that in females (Chapter 6). These results suggested selective influence on gonads of different sexes of the lizards. Adverse effects of thyroidectomy on androgen production by the male gonad is evidenced by degenerative changes in the testis and such changes are found in the epididymis also. However, in the females, ovary and oviduct do not show such response on thyroidectomy. On the basis of these facts it could be surmised that when thyroidectomy is performed and the rate of regeneration slows down in male lizards, the thyroid hormones directly and/or through their effects on gonads and the production of gonadal hormone influence the rate of tail regeneration in these lizards. To

substantiate further the aforesaid contention. of involvement of male hormone in regenerative process, testosterone was administered to thyroidectomised male and female lizards (Chapter 7). This treatment rectified the rate of tail regeneration to a certain extent in thyroidectomised males, while in females that got restored almost to its normal rate. Thus from the present study one can state that thyroid and gonadal hormones do play significant role in tail regeneration in these lizards. However, to understand the mode of action of these hormones influencing regeneration needs further studies.

From the study summarised herein, it becomes evident that changes occur in metabolic patterns in the visceral organs like liver, kidney; blood and adipose tissue and endocrine glands like thyroid and gonads during tail regeneration. Such systemic response to the regenerative activities in the lizard, H. flaviviridis indicates existence of provisions for changes in metabolic patterns to adapt and adjust to new morphophysiological demands that arise on autotomy and during regeneration.