Hemidactylus flaviviridis

# INTRODUCTION

As an organism's adaptive response to trauma following a loss of functional appendage, the phenomenon of reparative regeneration stands out between the realms of life and death. Eversince the time of Aristotle, scientists have been interested in this phenomenon. By definition, regeneration means rebirth or restoration of a lost part by one with almost the same physiological as well as morphological integrity as the original one, which may be in the form of cells, tissues, organs or large segments of the body. Restoration of the lost elements in living organisms during its day to day activity e.g. blood elements, uterine wall, hair etc., is termed as 'physiological regeneration'. While restoration of lost part caused by trauma is termed 'reparative regeneration'. The subject of regeneration has its depth as well as breadth. A wealth of data has accumulated on this fascinating phenomenon in last few decades. A number of excellent reviews are available on this subject (Morgan, 1901; Needham, 1952; Thornton, 1959; Hay, 1966; Schmidt, 1968; Goss, 1969; Rose, 1970; Weller, 1975 and Shah, 1975). Morgan (1901) introduced two basic terms for regeneration viz., morphallaxis in which residual tissues get reorganized to form the whole animal, however of a smaller dimensions, which is usually encountered in invertebrates as in hydra; whereas epimorphosis is the one by proliferation of the cells from the wound surface which is found in higher vertebrates e.g., limb regeneration in amphibians, tail regeneration in lizards, ear hole and antler regeneration in mammals. Thus the panorama of regenerative phenomena extends right from the most primitive coelenterates to highly evolved mammals. However, the selective forces of nature which exert the influence in perfecting various animal species have weighed the pros and cons of regenerative ability and bestowed it liberally only to less specialised organisms (Goss, 1969). For some animals regeneration is indispensible for the survival, while others have been left to get along without it. Nevertheless, with increasing complexity of organisms which are higher up in evolutionary tree, regenerative potential appears to become restricted to certain organs and appendages. Since many years the absence of universality of regenerative power has been a baffling problem for biologists. Nevertheless, since the turn of this century most of the animals that possess regenerative capacity have been tackled experimentally. A plethora of literature has amassed, and it is neither necessary nor possible to review all the investigations upto date. However, it will not be completely out of place to briefly outline the current state of our understanding of regeneration in vertebrates.

Schotté (1939) considered the process of regeneration in its broadest sense synonymous with reproduction. Schmidt (1968) emphasizes that these two processes <u>viz</u>., regeneration and development are analogous and not homologous as they differ in origin of the multipotential cells of the regeneration blastema and of the embryo. And also that regeneration depends on physiological stimuli while embryos develop autonomously. Goss (1969) and Bryant (1970) also have drawn similarities and dissimilarities between regeneration and embryonic development. Faber (1971) has elegantly drawn out morphogenetic parallels between vertebrate limb ontogeny and limb regeneration. Further it is really interesting to find that regenerative powers are greater in young forms and decline with increasing age (Hay, 1966). Contrary to this, Moffat and Bellairs (1964) and Bryant and Bellairs (1970) have pointed out that young lizard embryos do not have regenerative powers until they have reached a certain state of maturity.

The seemingly static organization of the adult organism from the point of view of extensive developmental processes is in fact deceptive, since cellular neoplasia is known to occur in almost all the adult organisms in response to a variety of stimuli. In recent years attempts have been made to draw out comparison between neoplasia and regeneration (Donaldson and Mason, 1975). Rose (1970) has made a very exciting proposal where he considered a tumour to be a case of never completed regeneration.

Goss and Grimes (1972) have made a clear distinction between wound healing and regeneration. Wound healing is so ubiquitous that any tissue in the body has got the power to heal itself, whereas regeneration is restricted to only a few parts of the body, if at all the animal has such potentiality. Goss (1972) has considered wound healing as an indispensible preliminary for regeneration. However, Maderson and Salthe (1969) reported that 'good' tail regeneration might equally be interpreted as a poorly controlled wound healing.

In reparative regeneration, formation of blastema - a mass of mesenchymatous cells covered by an epithelium - is an important primary event which quickly follows the wound healing of the exposed surface caused as a result of amputation or autotomy. In recent years investigations on epimorphic regeneration have largely centered on origin of blastema cell population (Trampusch and Harrebomée, 1965; Hay, 1966; Schmidt, 1968; Cox, 1969), potentiality of blastema (De Both, 1970; Michael and Faber, 1971) and autonomous self-regulating and organizing capacity of blastema (Faber, 1965; Stocum, 1968a & b). These studies have considerably extended our knowledge of morphogenesis in adult developing system.

Role of nerves in the regenerating appendages has also received considerable attention, especially the innervation of the appendage in question, and evaluation of a possible neurotrophic influence on the process (Zika and Singer, 1965; Singer, 1974; Geraudie and Singer, 1977; Jabaily and Singer, 1977).

Lizards represent the highest group of vertebrates that have retained considerable measure of regenerative ability, where autotomy and regeneration combined provide a means for escape and recovery from the assaults of predators. Tail regeneration in lizards is an unique example of regeneration; where the regenerated tail functionally however restored, its structure has been considered far from perfect. Since in such a tail nerve and muscles are atypical and cartilagenous axial skeleton does not segment or ossify (Woodland, 1920; Barber, 1944; Kamrin and Singer, 1955; Simpson, 1965; Cox, 1969). Bellairs (1969) and Avery (1970) have shown the importance of tail as a depot of lipids which may serve as an energy source during adaptive processes like hibernation and regeneration.

The morpho-physiological aspects of tail regeneration in lacertilians has been well accounted, but the phenomenon deserves intensive study in terms of systemic metabolic response to the tail autotomy and regeneration. Besides, the influence of hormones on this process also needs elucidation. The major changes that have attracted the attention of a number of investigators are those associated with repair and regeneration at the site of the damage and local metabolic adaptations in the regenerate. These studies have contributed considerably to our knowledge of lacertilian tail regeneration. To date, there have been a few studies indicative of systemic metabolic response in amphibian limb regeneration (Procaccini et al., 1971; 1973), but none in this regard in reptiles. Since regeneration involves replacement of the organ or appendage lost by one almost similar to that originally present, it is quite logical to anticipate excessive physiological demands on an animal as a whole in response to the stress following tail autotomy and its subsequent regeneration.

To obtain an insight into the physiological and biochemical changes taking place in various organs and tissues, along with the influence of hormones, the current investigations are designed to evaluate certain general physiological responses during tail regeneration in the house lizard, Hemidactylus flaviviridis.

The state of liver in terms of its metabolic makeup viewed from the syntheses and degradations of various metabolites during tail regeneration has been evaluated. Importance of hepatic glycogen as a major energy yielder in animals is well known. Shah and Chakko (1967) have reported glycogen to be the chief metabolite available in the normal and regnerating tail of H. flaviviridis. Hence, quantitative estimation of glycogen in liver, thigh muscle and corresponding levels of glucose in blood of this lizard during various phases of tail regeneration have been carried out. The importance of proteins in developmental processes has been widely recognised (Weber, 1967). Quantitative estimations of total proteins in tail and liver were carried out during different phases of tail regeneration. Lipid metabolism has been given considerable attention during tail regeneration in lizards. Although the role of lipids in energetics in vertebrates have long been realised, no evaluations of lipid contents of visceral organs have been reported so far during tail regeneration in the house lizard. The current investigations on lipids in liver and adipose tissue were undertaken to probe into the involvement of this metabolite in the regenerative process (Chapter 1).

Elucidation of the functional significance of an enzyme depends to a large extent on the knowledge of its localization and its position in the metabolic pathway. Significance of lactate dehydrogenase (LDH) as a regulatory factor in glycolysis (Somero, 1973) and that of $\alpha$-glycerophosphate dehydrogenase ($\alpha$-GPDH) in production of energy (Boxer and Shonk, 1960) are well known in animal tissues. Importance of the TCA cycle in energy yielding reactions of tissues is also well recognised. With these facts in mind, operation of anaerobic glycolysis and TCA cycle, and their participation in the metabolic processes of the liver during tail regeneration in *H. flaviviridis* have been evaluated by studying the activities of $\alpha$-GPDH, LDH, succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) histochemically (Chapter 2).

Some of the past and recent studies on the metabolism of vertebrate tissues have proved that ascorbic acid (AA) plays an important role in the carbohydrate, lipid and iron metabolisms (Banerjee and Ganguli, 1962; Rusch and Kline, 1941; Mazur *et al*., 1961). Hence, importance of this vitamin in the physiological processes underlying regenerative phenomena can not be ignored. Active participation of AA in collagen formation is well documented by Gould (1963). High content of AA was noticed by Shah *et al*. (1971) at the wound site in regenerating tail of *H.flaviviridis*. AA is known to be mobilised from distant organs during wound healing (Candlish and Chandra, 1967). To investigate similar possibility during tail regeneration, it was thought desirable to study the quantitative levels of AA in synthesising and storage organs of the lizard *viz*., kidney and liver respectively (Chapter 3).

It is only a matter of common knowledge that all the tissues and organ systems are connected intimately with each other through the medium of vascular fluids, hence it is to be expected that any change in physiological activities, especially those at the time of major reparative processes would be reflected in adaptive changes of the blood. Since practically nothing has been reported about such changes in the cell contents of blood during reparative regeneration in reptiles, it was thought worthwhile to obtain data on this aspect during tail regeneration in *H.flaviviridis*. To evaluate vascular response, total count of red blood corpuscles (RBC), white blood corpuscles (WBC), haemoglobin content and differential count of bone marrow cell population as well as of WBC were undertaken during tail regeneration (Chapter 4).

Significance of lymphocytes during wound healing is well established. Spleen being a major lymphoid organ in reptiles (Borysenko and Cooper, 1972), histological observations on spleen along with its gravimetric status were evaluated during tail regeneration in H. flaviviridis. This was further examined in the light of histochemical demonstration of hydrolytic enzymes such as acid and alkaline phosphatases. To evaluate further any potential involvement of spleen, splenectomy was performed during tail regeneration (Chapter 5).

Schotté (1926) emphasized the importance of hormones in regenerative process. In the last couple of decades, considerable attention has been given to the involvement of hormones in the regenerative processes of amphibians (Hall and Schotté, 1951; Schotté and Hall, 1952; Schotté and Chamberlain, 1955; Schotté and Bierman, 1956; Niwelinsky, 1958; Wilkerson, 1963; Waterman, 1965; Liversage, 1967; Tassava et al., 1968; Tassava, 1969;

Liversage and Scadding, 1969; Liversage and Livamagi, 1971; Schauble and Tyler, 1972; Liversage and Price, 1973; Vethamany-Globus and Liversage, 1973a & b; Schauble and Nentwig, 1974). Though some data on the influence of hormones on the process of regeneration are thus available, the results are often contradictory and difficult to interpret. There have been fewer studies suggesting the dependence of lizard tail regeneration on hormonal control (Licht, 1967; Licht and Jones, 1967; Licht and Howe, 1969; Turner and Tipton, 1971; Turner, 1972). However, the specific role of endocrine glands in the lizard tail regeneration is yet to be clarified. Changes in thyroid gland associated with tail regeneration in lizard, H. flaviviridis are studied in this laboratory by Shah and Chakko (1968b) and Magon (1970). Similarly Turner and Tipton (1971) suggested that normal tail regeneration in lizard, Anolis carolinensis is dependent in part upon circulating levels of thyroxine. With these facts in mind, it was thought desirable to study the effect of thyroid hormones on the rate of growth of the tail regenerate by extirpation of the gland. Influence of thyroid hormones on gonadal activities in lizards has been suggested by Eyeson (1970) and Plowman and Lynn (1973). In this context, it was felt necessary to evaluate the state of gonads in thyroidectomised lizards. Such a study would not only confirm the thyroid-gonad axis, but would also provide some insight on the influence of gonadal hormones on the regenerative process. For this, index of androgen activity has been examined by evaluating the cell heights of sexual segment of kidney and that of epididymis (Chapter 6).

The effect of gonadal hormones in lacertilian tail regeneration has been completely neglected. Androgens have been shown to promote an overall growth (Brody, 1945; Gaunt, 1954) and tissue regeneration in mammals (Joseph and Dyson, 1966; Dyson and Joseph, 1968). However, in lizard tail regeneration, no literature is available regarding the influence of androgens, which are of considerable physiological importance in reptiles through their effect on oxidative processes in their body (Chandola <u>et</u> <u>al</u>., 1974a & b). With these facts in mind, the influence of male hormone, testosterone on the regenerative process has been studied by administration of this hormone prior to autotomy (Chapter 7).