INTRODUCTION

The activity of an organ is the cause of its growth (Morgan, 1901). Years later, Boycott (1929) stressed that hypertrophy and atrophy of any organ are generally the use and disuse receptively. The greatest conceptual fallacy of all would be to look at differentiation which occurs in certain regenerating systems as a method by which the cells regain the developmental capacities of the undifferentiated polypotent cell. The concept of dedifferentiation, differentiation and polypotency have been studied in embryos and regenerating systems. "There are good grounds for supposing that the process of organ regeneration is merely an extension on a grander scale of the process of wound healing (Bullough and Laurence, 1966). Both are qualitatively similar phenomena and both are induced in the same way. The only difference between them is quantitative". Ambercrombie (1964) has suggested that "the raised incidence of mitosis and increased migration detectable in so many instances of wound healing, are in each instance set in motion by one stimulus; conditions suitable for the expression of cell migration and of mitosis are not identical, so that dissociation of the two processes may occur". "Regeneration is a repetition of ontogenesis, in so far as the organ districts involved are the same, but the processes are of necessity some what different (Needham, 1942). Rebirth, restoration of lost or damaged organs and parts and regeneration all implie the same. Regeneration is not only the resumption of temporarily arrested growth but also involves wound healing, differentiation and growth". "If there were no regeneration there could be no life, if every thing regenerated there could be no death" (Goss, 1969).

Regeneration, however, occurs in various degrees in plants and animals. It is now learned that many of the tailed amphibia can regenerate just about any part, if the removal of that part does not kill the organism before regeneration can take place i.e. tail, lens of the eye, major parts of the digestive tract, other visceral organs and parts of the head. Regeneration, like other biological processes is susceptile to analysis at various levels of organisation. While an attack on the fundamentals of the process should unquestionably begin at or near the molecular level and progress to more complex supramolecular situations, the absence of adequate methods compels us, instead to move down the historical avenue of approach dealing with superficially more simple natural units - the cells and tissues. The knowledge gained from such studies is basic only to an understanding of tissue and organ development and cannot provide information as to the more fundamental process of regeneration. However, any study of the physiology and biochemistry of regeneration will benefit from a clear statement of the morphologic events of the process. Contribution towards the morphologic, metabolic and biochemical aspects of regeneration were done by Okuneff (1933), Johnson and Singer (1964), Nivelinski (1958), Wolfe and Cohen (1963) and Schmidt (1968) in amphibians, and Radhakrishnan (1972), Hiradhar (1972) and Ramachandran (1972) in reptiles. Kinariwala (1977) and Kothari (1977) have given importance to the systemic factors involved in the process of regeneration. A comparative study of the biochemical changes taking place during regeneration of autotomised tail in Mabuya (Mabuya carinata, a regenerating type) and tail amputation of calotes (Calotes versicolor - a non-regenerating type) was done in this laboratory (Kinariwala, 1977). Though a clear cut biochemical difference was established between the two, it is important to extend such a comparative study to the adaptive responses occurring subsequent to loss of a regenerating part as opposed to that of a non-regenerating part in the same animal, in order to get a clear idea of the differences that are involved in the processes.

The Gekkonid lizard, Hemidactylus flaviviridis casts off its tail as an important self defensive mechanism to escape from its predator. After casting off the tail, a new tail regenerates which is more or less of the same length as the one prior to casting off, with some structural differences. Nevertheless, same lizard when looses its limb accidentally does not get a replacement. The most important puzzles in this process are self awareness that a part of its tail is cast off and the modus operandi involved in the transfer and translation of this awareness to functional restoration of the lost length of the tail. What are the factors involved in this? Why it is not possible with the limb? The answers to these questions are still not available.They may never be found unless we start worrying about the fundamentals. Each aspect helps in solving a microcosm of the puzzle and only if we look for the emerging patterns may we eventually see "the forest for the trees".

The study of the mechanics of wound healing and regeneration and the factors which control them began to arouse the curiosity of the scientists only during the beginning of the 20th century. It would be interesting to study the metabolic changes taking place during wound healing subsequent to loss of a part which does not regenerate as opposed to those taking place in response to autotomy of a part which usually regenerates and as such was undertaken. In this light, two sets of animals (Gekkonid lizard, _H. flaviviridis_), one with their tails autotomised (two to three segments away from the vent) and another whose limbs were amputated with a sharp blade from the distal part of the humerus served as experimental lizards. These two sets were studied for gaining a comparative idea of the adaptive physiological responses and their regulations characteristic of the two processes. Studies on reptilian regeneration have highlighted the importance of many physiological parameters of local as well as systemic sources such as blood glucose, tissue glycogen, protein, ascorbic acid and many enzymes (Shah and Chakko, 1967b; Radhakrishnan, 1972; Shah and Hiradhar, 1974; Shah _et al._, 1977a, b; Ramachandran _et al._, 1980; Swami, 1981; Ramachandran _et al._, 1975; Shah _et al._, 1976).

In this light, alterations in blood glucose and levels of glycogen, protein, ascorbic acid and activities of acid and alkaline phosphatase have been carried out at the local site and in liver and muscle post-caudal autotomy as well as post-limb amputation in _H. flaviviridis_ (Chapters 1-4). Evaluation of the above parameters have indicated a commonness under both conditions as far as the systemic responses are concerned. However, the local responses under the two conditions were found to be dissimilar, with the tail subsequent to its autotomy depicting changes which were not shown by the limb subsequent to its amputation. Moreover, the response of the tail subsequent to its autotomy and subsequent to limb amputation was differential, while,the limb responded in an identical fashion following its amputation as well as after caudal autotomy. These observations have led to the idea that an extremity capable of undergoing regeneration can evoke certain regeneration specific adaptive physiological and metabolic alterations while the extremity incapable of regeneration has some how lost this ability of adaptive physiological modulation.

In continuation to the above comparative evaluation, possible influence of autotomy induced state of the animal, on simultaneous amputation of a non-regenerating part was thought worthwhile to look into. In this wake, simultaneous to tail autotomy, limb amputation was also done to see whether under the pervading influence of tail regeneration, limb regeneration could be induced or under the stress of limb amputation tail regeneration could get retarded. Apart from the morphological observations, analysis of various physiological parameters in terms of metabolites and enzymes (as stated earlier i.e. Chapters 1-4) were also carried out under the double stress of tail autotomy and limb amputation (Chapter 5). Though the double stress of simultaneous tail autotomy and limb amputation induces some variations in the degree of systemic responses, the general pattern of changes was more or less identical to those obtained during tail autotomy or limb amputation alone. Apparently, under the double stress condition, the tail could show a normal process of regeneration, and the limb, however, again failed to show regenerative changes to any degree.

This has strengthened the idea that eventhough permissive systemic influences are in operation, the competence of the local site to tune with such systemic alterations is an important rationale which somehow for one reason or another is lost by the limb.

Vitamins are important cofactors in many metabolic and other processes. Of the various vitamins, vitamin C has been shown to undergo quantitative alterations during tail regeneration in lizards (Ramachandran et al., 1975; Shah et al., 1980). However, vitamin A has not been quantitatively assessed in relation to regeneration in vertebrates. Such an evaluation was deemed fit as vitamin A has been implicated in many events such as transcription, differentiation and morphogenesis (Tsai et al., 1978; Aydellote, 1963). Quantitative analysis undertaken in the present study showed positive modulations at the site of regeneration as well as in the liver and kidney. The increment in vitamin A content obtained in the study has been discussed in terms of its involvement in the number of biological activities concerning regeneration and wound healing.

Regulatory mechanisms involved in the secondarily reactivated events of development and differentiation as exemplified in the regeneratory process of vertebrate appendages have so far eluded human experimental excavations. Some of the explorations have however, met with minor success and many of the factors or agents in these experiments are mostly non-specific and only partially successful in, either initiating or controlling the process of regeneration. These agents vary from galvanic stimulation to saline treatment, vitamins to nucleoproteins and ependyma or nerve fibres to humoral factors. Though some of the endocrine factors are reported to be capable of influencing regeneration, the exact mode of action of the various hormonal factors is not yet fully explored. Some of the investigations conducted from this laboratory in the recent past have unequivocally demonstrated the involvement as well as adaptional alterations in the various systemic profiles of visceral origin in response to the stimulus of autotomy of reptilian tail.

In this light, a detailed investigation of the involvement of gonad and adrenal would give a useful insight into regulatory mechanisms of some of the endocrines involved in vertebrate regeneration. An interesting aspect that could be ascertained is the nature of involvement of the various endocrine factors as to whether direct, by acting at the local site of regeneration or indirect by bringing about subtle but effective alterations in the metabolic activities and other systemic factors. Considerable importance has been given to endocrine factors in regeneration especially in amphibians and fishes (Liversage and Price, 1973; Liversage et al., 1971), while in reptiles such aspects have received only limited attention (Turner and Tipton, 1971; Turner, 1972; Shah et al., 1979b; Kothari et al., 1979). The influence of androgens has been shown on gonopodium regeneration in certain teleostian fishes (Hopper, 1949 a,b) and ear hole regeneration in rabbits (Joseph and Dyson, 1966; Dyson and Joseph, 1968). Physiological importance is being attributed to sex hormones (androgens) due to their effect on oxidative metabolism in reptiles (Chandola et al., 1974 a,b). Because of this they were considered to influence tail regeneration in Hemidactylus flaviviridis (Shah et al., 1979 b; Kothari, et al., 1979). A delay in the formation of the blastema during non-breeding period was also taken to stress the importance of sex steroids during breeding season. Gonadectomy was conducted during breeding season to see the role played by the gonadal hormones during regeneration. Glycogen, protein, lipid and ascorbic acid were all shown to have profound influence in the biochemical and molecular mechanisms associated with the process of regeneration. It was therefore, considered important to study the changes, if any, in these parameters under gonadectomised condition. The data so obtained was compared with those from sham operated and intact normal lizards. Apart from local alterations, systemic alterations in these parameters were also looked into along with the rate of growth of the regenerate (Chapters 7-12).

The observations indicate that the normal tail elongation was not affected by gonadectomy. In fact, gonadectomy tended to induce a better regenerative growth till about the 25th day post-autotomy, though initially there was a 48 hr delay in the formation of a blastema. Apparently, gonadal steroids may be useful in faster healing, and absence of gonadal steroids could be beneficial in proliferation and differentiation of cells of the regenerate, basic fundamental requirements for regeneration. Moreover, the various parameters investigated also depicted a more or less identical pattern of modulations during tail regeneration in gonadectomised lizards as was observed for the controls. This once again suggested that the regeneration specific alterations in metabolites and enzymes are essentially mediated by humoral factors other than gonadal hormones.

On the basis of a series of experiments, Schotte and his associates (1961) postulated that pituitary adrenal synergism is the major controlling factor especially in the early stages of normal limb regeneration, which include wound healing and differentiation. Most of the work in this field was done on amphibians. To find out the role of adrenal, unilateral adrenalectomy was done, as a preliminary step towards this end, as bilateral adrenalectomy was found to be lethal to the animals. The ensuing changes, if any, on regeneration will have to be due to adrenal insufficiency. Sham operated and normal animals were also watched along with the operated ones. Levels of certain metabolites like blood glucose, glycogen, protein etc. together with certain enzymes and ascorbic acid were studied at the local site as well as systemically (liver, muscle) along with the rate of growth of the regenerating tail (Chapter 13). As in the case of complete gonadectomy, unilateral adrenalectomy also failed to retard regenerative ability or even to inhibit the expected physiological alterations. The only noticeable effect was during the first week of regeneration, whence not only the regenerative growth was retarded but even the physiological modulations were slow to respond. Obviously, the prevailing hypoadrenalism could be considered responsible for this. However, during the later periods, not only the rate of growth of the regenerate was as in the controls but even the various physiological parameters fell in line with the normal pattern. Apparently, the compensatory hypertrophy and hyperactivity of the remaining adrenal, (which was observed) enabled the lizards to bring about the normal process of regeneration. From the results it has been accrued that complete supression of both the adrenals could definitely hamper normal regeneration. This would indicate that adrenal principles may be involved directly or indirectly in supporting regeneration by regulating many adaptive physiological modulations.

Periods of study :

1. Caudal autotomy and limb amputation, comparative studies - breeding months (March - May)
2. Vit A - monsoon months (July and August)
3. Gonadectomy - breeding months (March - May)
4. Unilateral adrenalectomy - non-breeding months (December and January)