GENERAL CONSIDERATIONS

In the last two decades, enough biochemical and metabolic evidences have been amassed to emphasize the role of special molecular physiology and molecular ecology of the local site in amphibian limb and reptilian tail regeneration.
Many of these studies from our laboratory conducted on two lizards, *M. carinata* and *H. flaviviridis* have indicated altered metabolic profile to meet the energy requirements for the various events associated with regeneration. Apart from a metabolic shift from anaerobic to aerobic and then back to anaerobic, and alterations in ascorbic acid content involvement of many other factors such as neurotropic factors, proteins, cyclic nucleotides, neurotransmitters etc., have also been speculated to influence the process of regeneration at different stages and levels. The collective impression that can be gained from the various reports on vertebrate appendage regeneration is a frenzied but well synchronised biological activity that leads to the erection of a new structure (regenerated limb or tail) in place of the lost old one. It is well realised that in this process of reconstruction, many of the events unique to embryonic development (thought to be more or less shut off to a condition of suspended animation) are reawakened from their slumberous condition in a miniature version at a localised site. Since the power of regeneration is neither shown by one extremity in all vertebrates nor by

all extremities in one vertebrate, it is logical to pose the query of why some parts are capable of undergoing regeneration while others are not? Presumably, the only speculative answer that could be given are that either some extremities of the body in some vertebrates have retained certain factors essential for the above mentioned reawakening act or else only certain parts have retained the ability to respond to factors of reawakening. Apparently, the loss or masking off of such factors in either way could have led to the loss of regenerative ability in most of the vertebrates. Demonstrated influence of a threshold level of nerve fibres, neurotropic factors, cyclic nucleotides, simple salt solution, galvanic stimulation etc., may be relevant in this context and support the speculative suggestion that some parts have retained the ability to respond to factors of reawakening. Since the whole process of regeneration occurs in proximo-distal continuation of the stump tissues and also in association with the body itself, the curiosity to explore the probable participation or involvement of systemic factors of body origin was compelling. Though the process of regeneration is unquestionably a local event, our school of thoughts was focussed on an evaluation of the systemic responses, and accordingly, quite a voluminous literature has been compiled from such studies during the last six to eight years by making use of the lizards, *M. carinata* and *H. flaviviridis*. Results of such studies have proved interesting and encouraging much more indicative of significant systemic responses in conjunction with the local events. Though most of the observed body responses can be considered to bear a casual relationship with the process of regeneration, it is quite likely that some of the changes may be more synergestic and permissive than instructive. With the establishment of both local events and systemic responses, the stage was set to for appropriate questions to be asked to suitable valid animal models in pursuit of the possible reasons to the universally known restricted ability of regeneration among vertebrates. In an attempt to find suitable answers to this problem, comparable studies post-caudal amputation in Calotes versicolor, a lizard with no regenerative ability were carried out. When compared with the results obtained in Mabuya carinata after its tail autotomy, it was evident that the adaptive systemic alterations characteristic of Mabuya carinata during its tail regeneration was totally lacking in Calotes versicolor. Since in this case comparison was made between two different lizards, it would be more pertinent and valid to conduct such comparative studies on the same animal when two different extremities, one capable of regeneration and the other not, are amputated. Hence in the present study the response obtained during tail regeneration are compared with those obtained post-limb amputation (which does not regenerate) in H. flaviviridis.

Number of parameters like blood glucose level, hepatic and muscle glycogen contents, hepatic and muscle protein, hepatic and renal ascorbic acid contents and hepatic and muscle phosphatases were assessed. In contrast to the results obtained for Calotes versicolor, interestingly, the present studies revealed more or less identical systemic responses post-caudal autotomy as well as post-limb amputation. However, the in loco alterations were vastly different and the limb stump failed to depict the characteristic modulation typical of tail stump prior to and during its regeneration. Again, the cross responses of the limb and tail post-caudal autotomy and post-limb amputation respectively were also dissimilar with the result the responses of the limb post-caudal autotomy as well as its own amputation were more or less indistinguishable while those of the tail post-limb amputation as well as its own autotomy were very much at variance. Alterations in individual parameters are described and discussed in detail in chapters 1-4. The outcome of these studies is that some sort of a tentative conclusion could be drawn which serves to provide a preliminary answer to the initial query vis-a-vis restricted ability of regeneration. The picture that emerges in this respect is that the animals endowed with the power of regeneration retains the potential to evoke necessary systemic adjustments in response to the amputational or accidental loss of any body extremity.

However, the ability to regenerate the lost extremity depends essentially on the capacity of the local site to respond and react to in a fashion conducive for regeneration. Therefore, it appears that the capacity to regenerate a lost appendage in a vertebrate would depend on both the local potentiality as well as the ability to evoke specific synergistic, supportive and permissive systemic responses. Such an hypothesis if accepted would lead to further penetrating and searching experimental explorations in unravelling the still mysterious local factors on one hand and in determining the extent of dependance by the local site on various factors of systemic origin and segregating them into factors of absolute or optional importance on the other hand. Though the former may prove to be far from easy, the latter may be feasible by inhibiting specifically some of the observed systemic responses. Profitable future avenues of investigations may be undertaken on these lines.

In continuation of the above comparative evaluation, possible influence of autotomy (of a regenerating part : tail) induced state of the animal, on simultaneous amputation of a non-regenerating part (limb) was thought worthwhile to look into. Hence, simultaneous to tail autotomy, limb amputation was also performed to see whether under the pervading influence of tail regeneration, limb could be induced to regenerate or conversely, under the stress of limb amputation, tail regeneration could get retarded. Apart from morphological observations of the local sites, analysis of various physiological parameters in terms of metabolites and enzymes was carried out under the double stress of tail autotomy and limb amputation (Chapter 5). Such a study might provide further validity to the concept of a local factor hypothesis suggested earlier. Though the double stress of simultaneous tail autotomy and limb amputation induced 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. The fact that under the double stress condition the tail could show a normal process of regeneration and the limb no regeneration, has provided validity to the earlier conclusion of an inherent potentiality or ability to evoke certain regeneration-specific factors at the local site. A tentative hypothesis that could be suggested is that, whereas the tail has the necessary local potentiality, the limb does not, and a successful process of regeneration depends upon both adaptive systemic responses as well as the necessary local potentiality. Absence of any one of these factors could lead to loss of regenerative ability. Thus it appears that in an animal which is capable of regeneration of lost parts, systemic responses are well manifested, while the local potentialities are restricted only to certain regions of the body. Again it may be presumed that in animal which has lost the regenerative ability totally, both local potentiality as well as adaptive systemic responses characteristic of the process of regeneration are somehow masked. Since all biological functions are genetically determined, and the necessary information for all the activities is locked up in the molecular vault - the genome, it is illogical to think of loss of any biological function or activity. It is only likely that the evolutionary pressures might have brought about genetic and/or epigenetic masking of the process more completely in aves and mammals and incompletely in amphibians and reptiles. It is only logical that ways and means could be devised to unmask the masked components, regeneration could be successfully initiated in all the vertebrates with much practical significance in mankind. But as the proverbial saying goes, "there is many a slip between the cup and lip" and it is easy to bask in the glory of an imaginary world than turn it into a practical reality. With proper approaches and a bit of luck it may be in the years to come be possible to identify some of the masking agents or causes of masking and with still better luck and judicious approach lunicate ways and means of unmasking them.

From abstract thinking coming back to the present work done we continue the consideration of other factors viz. vitamins. As vitamin A has been considered to participate in many biological activities such as gene transcription, cell proliferation and differentiation, biosynthesis of glucosaminoglycans, glycolipids, glycoproteins, and membrane permeability and transport of ions and organic molecules (Bunting, 1967; 1969; Conard et al., 1974; Lazarus and Hatcher, 1975; Dusheiko et al., 1978; Ganguly et al., 1978; Tsai and Chytil, 1978; Stillwell and Rickett, 1980) and as also vitamin A treatment has been shown to promote post-amputational regenerative changes in the limb stumps of toad tadpoles by intensifying dedifferentiation and helping in the formation of a good blastema (Saxena and Niazi, 1971; Jangir and Niazi, 1978; Sharma and Niazi, 1979), a quantitative evaluation of vitamin A content in the regenerate, liver and kidney has been attempted (Chapter 6). This investigation has definitely shown a biphasic (pre-blastemic and post-blastemic) increase in vitamin A content in the regenerate and substantial accumulation and depletion of the vitamin stores in liver and kidney during the course of tail regeneration. Apparently vitamin A content of the body does depict a synergistic local and systemic modulation during the course of tail regeneration. In the wake of the earlier reported definite involvement of vitamin C in tail regeneration in lizards (Shah et al., 1976, 1980; Ramachandran et al., 1975), the present results on vitamin A gives added impetus to probe deeper into the roles of the vitamins as regulators of regenerative events.

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 exacavations. 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 varying from galvanic stimulation to saline treatment, vitamins to nucleoproteins, and ependyma to 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 tails.

In this light, a detailed investigation of the involvement of gonad and adrenal would give a useful insight into the regulatory mechanisms 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, 1973; Schmidt, 1968; Liversage et al., 1971), while in reptiles such aspects have received only limited attention (Turner and Tipton, 1971; Turner, 1972; Shah et al., 1979 b; Kothari et al., 1979; Shah and Chakko, 1968 a). The influence of androgens has been shown on gonopodium regeneration in certain teleostian fishes (Hopper, 1949 a,b), and earhole 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 (Chandola 1974 a,b) in reptiles. 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 the non-breeding period was also taken to stress the importance of sex steroids during the 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 significance 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. Though there were minor changes in various parameters studied, the general pattern tended to be similar in all the three groups of lizards during the process of tail regeneration. Though there was an initial 48 hr. delay in the formation of blastema, post-blastemic periods were marked by a better growth rate in the gonadectomised group of lizards as compared to the control groups. In the light of reports indicating the ability of steroid hormones to bring about faster wound healing (Roth <u>et al</u>., 1981) the initial delay observed currently in the pre-blastemic phase is understandable, However, the faster growth rate depicted by the gonadectomised lizards in the post-blastemic periods is suggestive of the fact that proliferative and differentive events are comparatively free of the influence of gonadal hormones. In fact, it appears that these events could occur better in the absence of gonadal steroids. Thyroid hormone is considered to be intimately involved in the post-blastemic differentiative events in lizards as per the previous observations from this laboratory. These two reports when taken together is suggestive of the possible antagonistic nature of gonadal steroids on thyroid hormone actions. It would be interesting in this context to investigate whether exogenous administration of gonadal hormones in the breeding months would bring about severe retardation in regenerative growth.

Another endocrine gland thought pertinent to investigate was the adrenal. Hypophysectomy had been shown to inhibit urodelian fore limb regeneration by severely affecting would healing (Schotte and Hall, 1952). It was reported that the role of pituitary in urodelian limb regeneration may be due to synergism with adrenal. In amphibian regeneration pituitary-adrenal synergism has been reported by a number of workers (Schotte and Linderberg, 1954; Schotte and Chamberlain, 1955; Schotte and Bierman, 1956; Liversage and Price, 1973; Bromley, 1977). 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. Since there is lack of information on involvement of adrenal in reptilian regeneration, 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 watched along with the operated ones. Levels of certain metabolites, like 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. As in the case of gonadectomised lizards, unilaterally adrenalectomised lizards also depicted a 24 hr. delay in the formation of blastema. Moreover, the initial alterations in various parameters noted to occur were at variance with those of control groups. The initial pattern of alterations characteristic of control-lizards were found to be delayed in the experimental lizards. These effects must in all probability be due to the insufficiency of the adrenal hormones rendered by unilateral adrenalectomy. The establishment of similar pattern of alterations in the various parameters (studied) comparable to those of the controls and also the attainment of normal growth rate in the post-blastemic periods in unilaterally adrenalectomised lizards bespeak of the compensatory hyperactivity of the intact adrenal. Morphological and histological observations of the remaining adrenal have given justification to this end. Unlike in the case of gonadectomy, only hemi-adrenalectomy was noted to induce more serious disturbances. However, these disturbances get rectified essentially due to the compensatory overproduction by the other intact adrenal. Expectantly, complete suppression of adrenal cortex by some chemical means could bring about complete suppression or even greater degree of inhibition of the process of tail regeneration in lizards. It may be worthwhile to probe further on those lines so that an involvement of adrenal in vertebrate regeneration may be more lucidly understood.