INTRODUCTION

Reparative regeneration, a fascinating phenomenon, is an organism's adaptive response following loss of functional appendage/part. It serves as an excellent model for understanding the novel principles and mechanics involved in the process of development. The process of regeneration embodies a conglomerate of morphological events such as healing of the wound, formation of a blastema, differentiation and growth of a new regenerate etc. These spectra of morphological changes are the result of multiple cellular events such as dedifferentiation, differentiation, growth, death etc., of cells and tissues, which are in turn controlled by underlying molecular and biochemical events. Most of these events of development are well knit and precisely synchronised and unfold as a well programmed set of changes during normal embryonic development. This unfolding of the programme in a precisely timed sequential order can well be visualised to occur in an independent fashion during normal ontogeny of an organism. However, the process of regenerative ontogeny of a part of an animal stands out in distinction to the normal ontogenic process, in the fact that instead of occuring as a totally independent event, the regenerative development of lost part of an animal occurs in structural association with the fully differentiated animal body and probably even

in functional association. Though the inbuilt genetic programme, may be considered to be reactivated during reparative regeneration for reconstitution of a more or less exact replica of the part lost, the structural and functional association with the body suggests a definite systemic involvement. This becomes all the more clear when viewed in the light that unlike the egg cell (which by itself is a highly specialized cell with all necessary store of nutritive materials, information molecules and a different genetic set up), the part to be regenerated is in the midst of a totally different environmental set up. In such a set up, there is a primary requisite for building up the necessary energy source, information molecules etc., and a precise conducive set up for the reactivation of the developmental events. And once initiated the conducive environment and conditions need to be maintained continuously for the successful completion of the process. It is in this venture that the regenerate like an orphan that is equipped with a rejuvenated genetic programme remains dependent on the systemic factors for guidelines and support althroughout for the various processes to occur in a synchronous fashion and sequential order. Few workers have made useful contributions towards the understanding of the metabolic and biochemical intricacies involved at the local site of regeneration (Okuneff, 1933; Niwelinski, 1958; Wolfe and Cohen, 1963; Johnson and Singer, 1964 and Schmidt, 1968, in amphibians and Ramachandran, 1972;

Hiradhar, 1972 and Radhakrishnan, 1972, in reptiles). However, there has been a clear cut lacuna as far as the involvement of systemic factors of the animal body is concerned. As the regenerate remains very much a part and parcel of the parent body, studies on these lines merit attention. It was in this wake, with an extensive literature already established on the metabolic features of the regenerate itself, that, investigations were initiated in this laboratory on systemic factors during tail regeneration in lizards. These studies (Kothari, 1977 and Kinariwala, 1977) have given a prima facie evidence to the definite systemic adjustments and alterations in response to the stimulus of tail autotomy and ensuing regeneration. These studies have definitely indicated adaptive systemic metabolic alterations and haemodynamic adjustments as significant features during lacertilian tail regeneration. Obviously, invoking of such systemic responses would require the mediatory influence of humoral factors. This factual conception has provided motivation for explorations on endocrine participation on lizard tail regeneration, and the experimental results reported in the present thesis on the probable 'causal-effect' relationship between tail regeneration and differing functional status of thyroid follow as a sequal to this. In fact the current status of literature available on hormonal regulation of vertebrate appendage regeneration is fragmentary and even

contradictory. Even of the restricted investigations carried out so far on this aspect, majority are with reference to amphibian limb regeneration (Liversage, 1967; Schmidt, 1968; Liversage and Scadding, 1969; Liversage and Price, 1973; Liversage and Brandes, 1977; Liversage and Korneluk, 1978). Apart from the few studies of Licht and Jones (1967), Shah and Chakko (1968), Turner and Tipton (1971), Turner (1972), Shah et al. (1979c), Kothari et al. (1979) and Ramachandran et al. (1980a, 1981), the field of endocrinology of tail regeneration in reptiles is very much barren. Besides, most of the above studies on amphibians and reptiles are singularly restricted to a correlation of a particular humoral factor with either the rate of growth or histomorphology of the regenerate. Since extensive metabolic and other changes have been noted to occur both in loco as well as systemically (due mainly to studies emanating from this laboratory) and as such changes are known to be brought about by hormonal factors in the normal course of an animal's activities, possible influence of hormones on regeneration, mediated via such physiological and biochemical alterations could well be considered feasible. Thyroxine which is considered to be a very important metabolic hormone having multiple effects has been screened in this context for its influence on tail regeneration in the Scincid lizard, Mabuya carinata. Accordingly, to have a better understanding of systemic response, and also to gain concrete and confirmative

evidences in favour of the earlier studies from our laboratory on one hand, and to gain insight about the role and possible modes of action of thyroid gland in lizard tail regeneration on the other, physiological and biochemical studies have been attempted presently both in normal as well as experimentally manipulated lizards.

As a priori, the rate of growth of the regenerate has been measured to make relevant and valid correlations. Since the present study extended to two different periods (late non-breeding and early breeding) and as the seasonal physiological variations are bound to influence the process of regeneration, the rate of growth of the regenerate during different phases of tail regeneration has been calculated from the length of the regenerate at fixed time intervals during both the late non-breeding as well as early-breeding periods. The total length of the tail regenerated at the end of 60 days though showing no difference during both the periods, however, does show a differential per day rate of growth. Though there was a gradual increase in the rate of growth in the first 15 days in both the cases, the rate of growth was perceptably higher during the late non-breeding period. Since then, from 15th to 40th day post-autotomy there was a fall in the rate of growth in both the groups of animals with the fall being more pronounced in those studied during their late non-breeding season. During the period between 25 to 40 days the rate of

growth was identical in both cases. During 40 to 60 days there was a further rise in the rate of growth but with a higher rate in the breeding period. The differential physiological and endocrine status could be considered to be responsible for the differential rate of growth observed at different stages of regeneration during the above specified two seasons.

Earlier studies from this laboratory have indicated increased metabolic alterations with metabolic shifts at the local site (regenerate) during tail regeneration in Mabuya carinata (Ramachandran, 1972 and Radhakrishnan, 1972). Liver and skeletal muscle being organs of immense importance in carbohydrate metabolism, it was deemed fit to study the quantitative changes in glycogen content as well as its degradatory enzyme glycogen phosphorylase in these two organs together with the regenerate during various periods of tail regeneration. The glycogen content in the liver and muscle was found to show a depletion during first fortnight of regeneration. Concomitant correspondence in phosphorylase activity was denoted by the mirror image pattern of changes of this enzyme with that of glycogen content during this period in the three organs. Corresponding gradual increase of glycogen content in the regenerate during the first 10 days post-autotomy with concomitant low level of phosphorylase activity are indications of mobilization of carbohydrate reserves from the systemic sources to the site of regeneration.

During the period of differentiation i.e., from 10 to 25 days the increasing level of phosphorylase activity and though high but declining level of glycogen in the tail regenerate are indications of active carbohydrate metabolism in operation there. During the later half of regeneration extending from 25 to 60 days the glycogen content as well as phosphorylase activity depicted changes directed towards return to the normal levels.

Having established a significant carbohydrate metabolism, LDH a key enzyme of carbohydrate catabolism and succinate INT reductase a marker enzyme of TCA cycle were deemed fit to study. The changes in the activity of these two enzymes in the three tissues under study indicate an increased anaerobic metabolism during the first 5 days (corresponding the wound healing period) and a pronounced aerobic metabolism during blastemic and differentiation phases of regeneration. The period after differentiation (i.e. 25 days onwards) is marked by a reverse trend of changes.

'Isozyme concept' put forward by Markert and Møller (1959) is gaining overwhelming application in various branches of Biology, especially in the field of Developmental Biology. LDH with its isozymic components is known to be capable of linking anaerobic breakdown of glycogen with aerobic oxidation through TCA cycle. In this light, with the purported operation

of a metabolic shift during tail regeneration in Mabuya (Shah et al., 1980 a) a detailed analysis of LDH in terms of its subunits and their concentrations as well as electrophoretic separation of its isozymes were thought relevant, during various periods of tail regeneration, in liver, muscle and tail. The results obtained have proved to be very resounding. Increased anaerobiosis during the immediate post-autotomy periods (upto 5th day) was indicated by the increased concentration of 'A' type of subunits of LDH and the appearance of pronounced LDH 5 and 4 (cathodic bands), isozymic bands. The period between 5th and 25th days post-autotomy which corresponds to blastemic and differentiation phases was marked by reduced 'A' type subunits, increased 'B' type subunits and appearance of anodic LDH 1 and 2 isozymic bands. Correspondingly there was also an increased analogue ratio (B/A). All these changes go definitely to prove the operation of aerobic metabolism during the blastemic and differentiation phases of regeneration not only in the regenerate but also in the liver and muscle. Return to the original anaerobic pattern of metabolism in all the three tissues is indicated by the gradual disappearance of LDH 1 and 2 isozymic bands, fall in the B type subunits and increase in A type subunits with corresponding fall in the analogue ratio. These changes commence after 25 days post-autotomy and slowly attain the original pre-autotomy levels by about the 60th day in all the three tissues under study.

Apart from their significance in protein synthesis, amino acids also contribute to general metabolism by acting as either oxidative substrates or as raw materials for lipid and carbohydrate synthesis. Since regeneration has been found to induce alterations with respect to carbohydrate, lipid as well as protein metabolisms, a quantitative evaluation of the total protein content in liver, muscle, serum and tail was undertaken during various stages of tail regeneration. Besides, Aspartate (GOT) and Alanine (GPT) aminotransferases, the two enzymes which are pivotaly placed to link amino acid metabolism with TCA cycle oxidations were also thought worth studying. These investigations have revealed increased positive nitrogen balance in all the three tissues as well as serum, especially upto 15 days of regeneration which corresponds to differentiation phase. The transaminases have also shown variations in all the tissues during regeneration. From the activity levels of these two enzymes in the tail it appears that these two enzymes are playing insignificant role in directing the TCA cycle intermediates towards amino acid synthesis. However, a glutamate centered amino acid metabolism appears to be more probable based on the activities of ICDH and GDH reported earlier from this laboratory in the same animal (Shah and Ramachandran, 1976 and Kinariwala, 1977). The principal source of amino acids during regeneration seems to be skeletal muscle as per the activity of the transaminases in this tissue. Liver also

seems to be playing a negligible role. Another source that might be playing an important role is the diet (i.e. cockroaches) which is a rich source of proteins.

Changes in levels of cyclic AMP in liver, muscle and regenerating tail during various phases of tail regeneration have been monitered indirectly by assaying the activity of cAMP phosphodiesterase, the only known degradatory enzyme of cAMP. During the first week of regeneration, (i.e. upto the formation of blastema) there was a significant decrease in the phosphodiesterase activity in the regenerate, followed by an increase upto 25th day. On day 40, there was again a significant fall, and thereafter, by 60th day it settled down to the normal level. These changes can be correlated with the changing levels of cAMP associated with wound healing, proliferation, differentiation and growth. Changes in the concentration of this enzyme in liver are statistically insignificant. Interestingly in the case of muscle, there were two phases of significant decrease; first one during the first 5 days and a second decrease during 25th to 60 days post-autotomy. These observations denote that muscle may be using the phosphodiesterase activity to control the cAMP concentration where as in liver, there may not be any change in the cAMP content or if at all these changes are probably under the control of adenylate cyclase, during tail regeneration in Mabuya carinata.

Of the various factors for a successful regeneration the neurotrophic factors are considered to be significant. Singer and his colleagues (Singer, 1952) have equivocally reported that denervation retards the regenerative capacity and they further emphasized the trophic factors released by central nervous system to be helpful in the regenerative ability. Keeping these facts in mind, in order to evaluate the involvement of nervous system, quantitative evaluation of total (ChE), acetyl (AChE) and Non-specific (N-SpChE) cholinesterases were carried out in the regenerate, liver and skeletal muscle. The level of ChE and AChE in all the three tissues depicted a parallel decrease during wound healing and pre-blastemic phases and a parallel maximal level during early differentiation phase. Since then by a gradual decrease during the late differentiation and growth phases, it settled down to normal levels by the time when the tail was fully regenerated. A similar pattern was depicted by N-SpChE too, except for the tremendous increase noted during 3rd day post-autotomy. This nine fold increase in N-SpChE activity is purported to be somehow involved (directly or indirectly) in the as yet unknown mysterious events associated with the process of initiation of regeneration. During the early differentiation period also N-SpChE showed elevated levels which could be correlated with lipid metabolism. Peak level of AChE may be correlated with metabolic alterations,

macromolecular synthesis and membrane permeability changes probably by bringing about a regeneration specific ionic flux.

Having established the significant involvement of the body as a whole in the regenerative mechanics, endocrine participation can be easily surmised as a corollary. In this respect our observations of an increased aerobic metabolism pervading the body at large, and the biphasic depletion of follicle content from the thyroid follicles noted (Ramachandran et al., 1981) during tail regeneration in Mabuya carinata, prompted detailed investigations directed at an understanding of the direct/indirect involvement of thyroxine during regeneration. Three groups of experimental animals were made use of for the purpose with one group of animals (Group B) being chemically thyroidectomised by force feeding with 0.1 ml of 0.1% solution of propyl thiouracil (PTU) on every alternate day commencing from four weeks prior to tail autotomy and thereafter. The second group of animals (Group A) were fed with an equal volume of distilled water and served as the controls. The third group of animals (Group C) were also force fed with PTU, like that of group B and were subjected to replacement therapy by giving 1 µg/gm body wt. of thyroxine (obtained from Sigma Chemicals, USA) intraperitonially every alternate day commencing from 20th day after PTU treatment. Since then both PTU treatment as well as intraperitonial injections of thyroxine were

continued upto autotomy as well as thereafter. After tail autotomy the blood of all the three groups of animals were analysed for changes in levels of blood glucose. Further, changes in the levels of glycogen, protein, phosphorylase, succinate INT reductase, transaminases, and phosphodiesterase were also assayed in the tail regenerate as well as liver and skeletal muscle. Besides, alterations in ascorbic acid content of liver and kidney were also evaluated.

The studies on rate of growth of the regenerate show a tremendous retardation in regenerative ability of hypothyroidic animals to the extent of about 71% as compared to the control animals. In the group of animals subjected to replacement therapy there was a significant recovery in the rate of growth and was behind the control group only by about 24% by the 60th day post-autotomy. In the hypothyroidic group of animals, the regenerates did not proceed beyond the late blastema stage. Moreover, the quality and size of the blastema formed were also very poor. A detailed discussion on these observations is given in Chapter I.

Carbohydrate metabolism which was reported to play a prominent role during tail regeneration in Mabuya carinata and which was observed to be the case in the control animals of the present experimental set up was rendered inoperative due to chemically rendered athyroidic state. Depletion in

glycogen levels of liver and muscle and elevated level of blood glucose characteristic of early phases of regeneration lasting upto 15 days (differentiation phase) were less prominent in hypothyroidic animals. Correspondingly the phosphorylase activity was also affected. Animals put under replacement therapy showed significant changes in carbohydrate metabolism more comparable to the control animals. The tail regenerate also showed poor carbohydrate metabolism in PTU treated animals as compared to the control and T4 supplemented animals.

Normal process of regeneration is marked by increased aerobic metabolism which is reflected in the increased succinate INT reductase activity in liver, muscle and tail. However, on treatment with PTU such animals were seen to depict an altered succino-oxidase activity. Changes similar to control animals could be brought about by replacement therapy with T4. It follows from these observations that the inability to raise up the level of oxidative metabolism might be one of the factors responsible for the observed inhibition of regeneration.

As protein metabolism is of vital significance in developmental processes and as regeneration induces positive nitrogen balance in the body as noted by the increased levels of protein in liver, skeletal muscle and the regenerate during

normal regeneration as cited earlier, changes in the protein content of liver, muscle and regenerate were evaluated in experimental PTU treated animals as well as those animals which were given T4 simultaneously and compared with the corresponding control animals. Concurrently activity levels of GOT and GPT were also assayed in all the three tissues. The definite pattern of protein anabolism characteristic of normal regeneration was apparently upset in liver and muscle while in the regenerate it was significantly abolished. In the T4 treated group the changes were more comparable with the control ones. With reference to transaminases, functional athyroidism does not seem to have much influence. In all the three groups of animals, the pattern of changes appear to be more or less similar except for the sharper modulations in the PTU treated and PTU + T4 treated groups of animals. As increased levels of transaminases are considered to be indicative of tissue dysfunctioning, favouring protein catabolism, it is safe to presume that during regeneration protein catabolism is effectively prevented and anabolism was favoured as the activity of the transaminases did not register supranormal levels at any stage during normal tail regeneration. This could ensure the availability of sufficient amount of amino acids for the formation of new tissues during regeneration.

Cyclic AMP phosphodiesterase was studied as an indirect mode of understanding the involvement of cAMP during regeneration and its possible effect due to chemical thyroidectomy. An interesting feature is the persistant high level of the enzyme under hypothyroidic conditions in liver, muscle as well as the regenerate. Changes in the levels of phosphodiesterase in all the three tissues of the control animals were very much identical to those observed during normal tail regeneration during late non-breeding period as mentioned earlier. Animals subjected to replacement therapy appeared to show patterns of enzyme changes closer to those of the controls. Animals of group B which were rendered functionally athyroidic, though showed highly elevated levels of phosphodiesterase activity at all stages, did, nevertheless, depict modulations during different phases of regeneration, thus indicating the involvement of other humoral and/or control factors. However, the modulations exhibited by functionally athyroidic animals were out of step in comparison to the control and T4 treated animals as they were noted to occur either earlier or late during the time scale of regeneration. Significantly, the higher levels of the enzyme also indicate comparatively low levels of cAMP in group B animals, which might be considered to affect many biochemical activities so crucial for a successful process of regeneration.

Finally, levels of ascorbic acid in liver and kidney were also assayed in all the three groups of animals as ascorbic acid is known to be well involved during specific phases of regeneration. Once again as reported earlier (Shah et al., 1980 c) hepatic ascorbic acid appears to be less involved as compared to the renal ascorbic acid. A notable feature is a more or less parallel pattern of changes shown by both hepatic as well as renal ascorbic acid in PTU treated (Group B) animals. Renal synthesis and release of ascorbic acid appeared to show a different pattern between group A (control) and group C (PTU + T4 administered) animals. However, the hepatic ascorbic acid changes were more or less of the same pattern in both groups except for the fact that the modulations appeared to be slightly late in group C animals. Non-utilization of ascorbic acid in hypothyroidic (Group B) animals can be visualized by the increasing levels of ascorbic acid during the first 25 days post-autotomy.

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