GENERAL CONSIDERATIONS

Extensive local histophysiological alterations have been reported during reparative regeneration of lacertilian tail (see Chakko, 1968; Magon, 1970; Ramachandran, 1972; Hiradhar, 1972; Radhakrishnan, 1972). Metabolic transformations involving a switch over from an anaerobic, carbohydrate dependent type, to an aerobic, lipid dependent type, and its reversal as well as differential operation of glycolytic pathway, shunt pathway and TCA cycle oxidation, were elucidated by the above workers. Functional changes in the levels of sodium and potassium ions and the vitamin C, ascorbic acid were also reported to occur along with the progression of tail regeneration. Local occurrence of a conglomerate of multiple events such as macromolecule synthesis, metabolic interconversions, orderly energy channelization etc., within the regenerating system of an adult vertebrate appendage, though apparently similar to that of a developing embryonic system, cannot however, be considered parallel as the ontogenic system unlike the regenerating system is a store-house of information molecules, regulatory factors etc., which are already precisely programmed and pre-conceived. It was this obvious fundamental difference which led to the experimental exploration of systemic responses to the stress of regeneration in lizards. It is logical to expect such responses as the regenerating

system pursues a course of re-enactment of ontogenic events, remaining very much as a part and parcel of the adult animal body. Some of the preliminary investigations carried out in this light have given excellent results (Kothari, 1977; Kinariwala, 1977). Evaluation of various parameters like levels of metabolites and nucleic acids in liver, levels of ascorbic acid in liver and kidney, levels of glucose, cholesterol and total lipids in blood as well as haemopoitic responses carried out in the above studies have indicated an effective participation of visceral factors of systemic origin in supplementing the local efforts during tail regeneration in lizards. Accordingly, hepatic glycogenolysis in the immediate post-autotomy periods, involvement of blood glucose as a source of metabolic energy for the regeneration blastema, pronounced lipid utilization and prevalence of an aerobic environment (both in loco as well as systemically) during blastemic and differentiation phases of tail regeneration as well as adaptive haematologic haemodynamic adjustments marked by haemopoietic changes and systemic ascorbic acid turnover were all inferred. In this light, necessity to explore further systemic responses together with the probable endocrine regulation of such alterations was felt. The present bipronged study is an offset of this thinking and involves coordinate biochemical analysis of many physiological parameters (in loco and systemically) on

one hand, and alterations if any, on some of these parameters under altered functional status of thyroid gland and its correlation vis-a-vis tail regeneration on the other hand.

The first part of the thesis deals primarily with physiology of regeneration involving aspects of carbohydrate and protein metabolisms, enzyme modulations in metabolic adaptations, role of cAMP and neurotransmitters, and morphological evaluation of tail regeneration in terms of breeding status and thyroid influence. The last aspect which forms the subject matter of chapter - I has revealed no apparent significant variation in tail elongation between late non-breeding and early breeding periods. However, a better rate of growth involving wound closure, blastema formation and commencement of histodifferentiation has been recorded during the late non-breeding period while rate of growth involving late differentiation and growth events has been noticeably better during the early breeding period. It is likely that the slightly lesser growth rate of early phases of tail regeneration during the recrudescent period could be due to the inability of the animal to direct its full metabolic potential in this direction as it has already been activated to meet the energy requirements of the ensuing breeding activities. In this light it would be pertinent to direct future lines of investigations on animals which are experimentally subjected to metabolic activation and their

ability to undergo early phases of regeneration such as wound closure, blastema formation and commencement of differentiation. The current observations also indicate the positive influence of reproductive hormones in speeding up of the process of differentiation and growth once the regenerate has reached the post-blastemic stage. Clarification on these lines also might prove fruitful. Thyroid influence on morphology of tail regeneration has depicted a clear cut inhibition of tail regeneration under functionally induced athyroidism. This when viewed in the light of the noticeable suppression of regeneration induced physiological responses of euthyroidic animals tends to indicate the indirect influence of thyroxine in supporting tail regeneration. However, the direct role of thyroxine can be negated only by studies involving local application of thyroxine as opposed to systemic T4 administration in hypothyroidic animals.

After having observed significant metabolic alterations locally, affecting carbohydrates, lipids and proteins during lacertilian tail regeneration (Chakko, 1968; Magon, 1970; Ramachandran, 1972; Hiradhar, 1972; Radhakrishnan, 1972), it was realized that some sort of systemic participation would be required to meet the energy requirements as well as the building up of metabolic reserves of the regenerate. This is pertinent in the sense that the regenerate unlike the

developing egg is not equipped with a ready source of metabolic energy. Some of the later studies initiated in this wake (Kothari, 1977; Kinariwala, 1977) have demonstrated involvement of hepatic glycogen and protein and adipose tissue lipids during caudal regeneration in H. flaviviridis and M. carinata. Present studies (Chapters II to V and VII to X) have not only confirmed the above findings but have also enabled in extending our understanding on systemic participation by demonstrating marked involvement of skeletal muscle as well as thyroid hormone. Some of the tentative conclusions that have been drawn from these studies are : (1) There is a biphasic hepatic glycogenolysis: once during the first five days and once during the 15th to 25th days of tail regeneration, with a period of glycogenesis in between. (2) There is a protracted muscle glycogenolysis lasting upto 25 days of tail regeneration. (3) There is an elevation in the blood glucose level during blastemic and early differentiation phases. (4) There is a positive nitrogen balance denoted by increasing hepatic and muscle protein contents. (5) There is increased anaerobiosis during the first few days post-autotomy followed by generalized aerobic environment during blastemic and differentiation phases of regeneration. Whereas hepatic glycogenolysis is considered to serve as an energy source as well as, a source for lipogenesis in the regenerate via blood glucose,

muscle glycogen is surmised to be important in hepatic gluconeogenesis as well as, a generalized source for amino acid biogenesis.

Alterations in the activities of muscle phosphorylase, LDH, as well as transaminases are convincing evidences in favour of this contention. Subnormal levels of transaminases in the regenerate all throughout regeneration indicate the inability to divert glycolytic and TCA cycle intermediates towards amino acid synthesis, though GDH mediated amino acid synthesis is considered a distinct possibility. However, a definite dependence on systemic sources for amino acids is inferred. The prevalence of aerobic environment during blastemic and differentiation phases of regeneration is confirmed by the observed coordinate increase in LDH 'B' subunits as well as the analogue ratio and the appearance of prominent LDH 1 and 2 isozymic bands in liver, skeletal muscle as well as the regenerate. Further evidence is provided by the observable increased SDH activity during these phases of regeneration. Involvement of thyroxine in the regenerative process is denoted by the observable inhibition of regeneration as well as the abolition of many of the systemic metabolic alterations outlined above in 6-propyl, 2-thiouracil (PTU) induced hypothyroidism (affecting hepatic glycogen and protein, blood glucose and muscle glycogen and protein) characteristic of euthyroidic

animals. This probably underscores the indirect mode of thyroxine mediation in the mechanics of tail regeneration in M. carinata.

Restoration of these features by T4 administration to functionally rendered athyroidic animals gives added validity to the concept of systemic participation in lizard tail regeneration. Further studies involving experimental manipulation of blood sugar level, hepatic and muscle glycogen contents etc., with pancreatic and adrenal hormones in both euthyroidic as well as hypothyroidic animals would not only provide information regarding the relative importance of one or more of these systemic metabolic alterations in initiation and progression of regeneration, but also bring out the role of thyroxine and/or other hormones in regulating the systemic metabolic adaptations.

Cyclic AMP has been implicated in many cellular events (Rall et al., 1957; Beriz et al., 1973; Jabaily et al., 1975; Short et al., 1975; Carlone and Foret, 1979; Kosher and Savage, 1980). An evaluation of cAMP participation in tail regeneration by way of an indirect method involving quantitative assay of cAMP phosphodiesterase (PDE) has also given evidences in favour of adaptive regulation in the levels of cAMP both systemically as well as locally. These alterations are correlated with events such as cell division and differentiation at the local site as well as regulation of functionally

potent levels of cAMP during tail regeneration by its influence on phosphodiesterase activity (Chapters VIII and XI). Since there are no studies on hormonal control of phosphodiesterase activity, detailed studies involving thyroid, phosphodiesterase activity, carbohydrate metabolism and tail regeneration would prove to be worth while an exercise in bringing out the intricate interrelationships that may be inherent in the above aspects. The present tentative surmise of a negative regulatory influence of thyroxine on PDE also needs further investigation.

The experimental findings outlined in Chapter VII, clearly indicate definite functional involvement of cholinesterases (ChEs) either directly or indirectly in tail regeneration. The most significant observation of a nine fold increase in non-specific cholinesterase (NspChE) locally in the immediate post-autotomy period (3 days) has proved to be a very interesting observation in the light of the reported roles and involvement of as yet unidentified neurotrophic principles of protein nature (Singer et al., 1976). The possible release of NspChE by the cut end of the spinal cord and its role in inducing a favourable conducive environment for the initiation of the process of regeneration (by bringing about alterations in membrane permeability, ionic fluxes etc.) cannot be overruled and as such needs further searching experimentation. Besides, the source of origin of this NspChE either from

the distal end of the spinal cord or the brain also needs to be ascertained. Since thyroxine is known to be very intimately associated with development and differentiation of the nervous system, the possible relationship of thyroxine with the currently noted local increase of NspChE after caudal autotomy is also a pertinent topic of future investigation.

Finally, the present investigations also confirmed the earlier report (Shah et al., 1980 c) regarding the active involvement of hepatic and renal ascorbic acid in meeting the local requirements of vitamin C during tail regeneration in M. carinata as shown by the alterations in the euthyroidic animals (Chapter XII). Though functional athyroidism per se does not seem to be affecting the regeneration associated systemic turnover of ascorbic acid, functional athyroidism does however, affect purposeful modulations as well as probably the utilization of ascorbic acid by the peripheral tissues. Future lines of investigations in this light might not only help in understanding the thyroxine regulation of ascorbic acid release and utilization but also throw more light on the relationship between thyroid, ascorbic acid and tail regeneration in lizards.

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