biochemical aspects of tail regeneration, with special emphasis on the pattern of metabolism.

The present investigations were carried out with a view to understand the metabolic intricacies underlying the phenomenon of tail regeneration in the Scincid lizard, *Mabuya carinata*. The investigations deal with both the histomorphological as well as the metabolic aspects of this fascinating phenomenon. Since these aspects have been treated as individual topics of discussion, in separate chapters in the thesis, it is thought necessary to piece together all these findings and present a complete generalized picture.

The histomorphological studies on the normal and regenerating tail of *Mabuya carinata* revealed certain interesting features, when compared with *Hemidactylus flaviviridis*. The anatomical architecture of the tail of *Mabuya carinata* seems to be unique; especially regarding the muscle arrangement. Considerable difference in scale pattern and structure and the dermal elements exist between the two lizards. However, both the lizards, revealed certain identical anatomical features especially the intervertebral autotomy plane. The exact origin and insertion patterns of the caudal muscles are worth investigating in that it might provide enough clues as to the similarities and or dissimilarities of the musculature of regenerating and nonregenerating lizard tails.

The histochemical investigations on the distribution pattern of the metabolites such as lipids and glycogen and the enzymes associated with aerobic and anaerobic metabolism revealed a metabolic flux with regard to the preferential choice of metabolites, in the regenerating tissues of the tail of Mabuya carinata. Carbohydrate seems to be the selective metabolite in the tissues of the normal tail of Mabuya carinata. This aspect is well exemplified by the present histochemically observed glycogen and phosphorylase (Chapter 3), aldolase and LDH (Shah and Ramachandran, 1972, 1970) and alpha glycerophosphate dehydrogenase (Ramachandran, 1972) in contrast to the reported poor localization of lipids, lipase and esterase (Chapter 2), betahydroxybutyrate dehydrogenase (BDH) and other TCA cycle enzymes such as SDH and ICDH, together with a poor response of glucose-6-phosphate dehydrogenase (G6PDH) thus representing an inadequate machinery for lipid catabolism (Ramachandran, 1972).

The presently observed glycogen and phosphorylase (Chapter 3) and poor levels of lipids, lipase and esterase activities (Chapter 2) in the normal tail tissues are indicative of an active anaerobic glyconeogenesis. Similar pattern of distribution of lipids and glycogen and associated enzymes were observed by Shah and Chakko (1967b, 1969), Chakko (1967) and Magon (1970) in the normal tail tissues of the house lizard, Hemidactylus flaviviridis. Considering all these facts it could be safely presumed that the tissues of the normal tails of both the lizards studied in this laboratory viz., Mabuya carinata and Hemidactylus flaviviridis have a similar pattern of anaerobic metabolism and a suppressed low levels of aerobic lipid utilizations. It becomes rather pertinent at this stage to query as to whether the subcutaneous and submuscular adipose tissues identifiable in both Mabuya carinata and Hemidactylus flaviviridis are merely for maintaining the structural integrity and shape of the tails or whether they are of some importance during hibernation and aestivation, or, is it also possible that they are mere store houses (in addition to the visceral fat bodies) for storing the excess fat present in the body of these lizards?

From wound healing onwards, through preblastema and blastema upto early differentiation phase a gradual progress of lipogenesis was the main feature noticed in the regenerating tail of Mabuya carinata (Chapter 2). Similar observations were reported by Chakko (1967) in the regenerating tail of Hemidactylus flaviviridis. At the same time a concomitant depletion of glycogen from original tail stump tissues was also observed from wound healing to preblastema; while glycogen and phosphorylase were noted to be poorly localized in the blastema (Chapter 3). Identical reports are available from the studies of Shah and Chakko (1967b) during early phases of regeneration in the tail of the house lizard, Hemidactylus flaviviridis. A progressive increase in the level of lipids seem to be the characteristic feature during the early phases of regeneration in both the lizards studied. The increasing activity of G6PDH, malic enzyme and ICDH reported by Ramachandran (1972) and Magon (1970) in the blastemic phases of Mabuya carinata and Hemidactylus flaviviridis respectively, are in good correlation in this perspective, as these enzymes are responsible for the supply of $NADPH_2$, an important cofactor for lipogenesis. The concomitant depletion of glycogen and a moderate activity of phosphorylase (Chapter 3) and the high activities of the chief glycolytic enzymes reported by Ramachandran (1972) from wound healing to blastemic phase tend to signify the diversion of glycolytic intermediary products towards lipogenesis. The persistent high activity of aldolase (Shah and Ramachandran, 1972) in the blastemic phase and the concomitant increased levels of α-GPDH (Ramachandran, 1972) lead to surmise that these two enzymes together might be playing a significant role in the synthesis of glycerides, and thus may be correlated with the increased levels of phospholipids at this phase. A strong evidence for the presently observed acidic lipids (Phospholipids) in *Mabuya carinata* during this phase (Chapter 2) is well supported by the studies of Kennedy (1957a & b) regarding phospholipogenesis from glycerophosphate produced by α-GPDH catalysis in conjunction with glycolysis. Similar observations of increased level of lipids (Chakko, 1967) and α-GPDH (Magon, 1970) were made, in the blastemic phase of the tail regenerate in the house lizard, *Hemidactylus flaviviridis.*

The production of pentose sugars and amino acids are again of utmost importance during blastema and early differentiation phases, as these products are of vital importance for the process of cell division and proliferation, which form one of the main events of the regenerative process. The increased levels of nucleic acids during wound healing and blastema phases (Chapter 6) are well correlated with the increased levels of G6PDH, the key enzyme of HMP shunt path way, which serves as the principal source of pentose sugars, much needed for the synthesis of nucleotides, nucleosides and nucleic acids. Since it is a known fact that α-ketoglutarate is a very important source for a number of amino acids and is formed as a result of ICDH catalysis, the moderate activity of ICDH reported by Ramachandran (1972) and a parallel increased level of nucleic acids during blastema phase is rather self explanatory. In short, one can predict that the significant role of these increased levels of lipids, nucleic acids and proteins is for the attainment of self sufficiency for the much higher and hectic events of the next phase to follow after the blastemic phase, the differentiation phase which is marked by very active rate of cellular proliferation and differentiation. In general, it may be presumed that the period between wound healing and blastema is an active phase and the ensuing differentiation phase, a period of catabolic activities.

As the differentiation progresses, from the late blastemic phase, almost all the tissues of the regenerate revealed a concomitant spurt in the activities of the presently studied enzymes, such as lipase and esterase (Chapter 2), phosphorylase (Chapter 3), aldolase, LDH, and MDH (Shah and Ramachandran, 1972, 1970), malic enzyme, G6PDH, BDH, α-GPDH, SDH, ICDH and Diaphorases (NAD.D & NADP.D), (Ramachandran, 1972 and Table 9.1), in <u>Mabuya carinata</u>. Identical reports regarding above listed enzymes also available from the studies of Magon (1970) on the differentiating tail of the house lizard, <u>Hemidactylus flaviviridis</u>. Stepped up activities of SDH and malic enzyme have been reported by Shah and Chakko (1969) and Hiradhar (1972) respectively in the same lizard, during the differentiation phase of tail regeneration. The presently reported decreased lipid content and a concomitant increased activities of lipase and esterase (Chapter 2) and other enzymes of

TABLE 9.1

Comparison of the concentrations of metabolites and certain enzymes represented in the normal and regenerating tail of the Scincid lizard, *Mabuya carinata*

Metabolites and enzymes	NORMAL TAIL		REGENERATING TAIL					FULLY REGENERATED TAIL	
	Epidermis	Muscles	Wound healing phase	Blastema phase	Early differentiation phase	Late differentiation phase	Growth phase	Epidermis	Muscles
Glycogen	++	+++	++	+	+++	++++	+++	++	+++
Phosphorylase	++	+++	++	+	+++	++++	+++	++	+++
@Aldolase	++	+++	++	++	+++	++++	+++	++	+++
**α-glycero phosphate dehydrogenase (α GPDH)	++	++	++	++	++++	+++	++	++	++
*Lactate dehydrogenase (LDH)	++	+++	++	+++	+++	++++	+++	++	++
*Malate dehydrogenase (MDH)	+	++	++	++	+++	++++	++	+	++
Lipid	+	+	++	+++	++++	+++	++	+	+
Lipase	+	+	+	++	+++	+++	++	+	+
Esterase	+	+	++	+++	++++	++++	+++	+	+
**B Hydroxy butyrate dehydrogenase (BDH)	+	+	-	++	+++	++++	++	+	+
**Succinate dehydrogenase (SDH)	±	±	±	+	+++	+++	+	±	±
**Isocitrate dehydrogenase (ICDH)	±	±	+	++	++++	+++	++	±	±
**Glucose-6-phosphate dehydrogenase (G6PDH)	++	++	+++	+++	++++	+++	++	++	++
**Malic enzyme	±	±	+	+++	++++	+++	+	±	±
**Cytochrome oxidase	±	±	±	±	++	++	±	±	±
**NAD-Diaphorase	+	+	+	++	++++	+++	++	+	+
**NADP-Diaphorase	±	±	±	+	+++	++	+	±	±
Ascorbic acid (AA)	+	+	++	+	++++	++++	++	+	+
Acid phosphatase	++	=	++	+++	++	++	++	++	=
Alkaline phosphatase	++	++	+	++	+++	++++	++	++	++
Nucleic acids (DNA & RNA)	+	++	++	+++	++++	++++	++	+	++
Acetylcholinesterase (AChE)	+	+++	±	±	++	+++	++	+	++
Butyrylcholinesterase (BuChE)	+	++	±	±	++	+++	++	+	+

++++ very high; ++ high; ++ moderate; + poor; ± negligible; = absent.
@ Shah and Ramachandran (1972)
* Shah and Ramchandran (1970)
** Ramchandran (1977)

TCA cycle, *viz.*, SDH, ICDH together with BDH are suggestive of the lipid utilization during this phase.

Persistent activities of LDH and MDH (Shah and Ramachandran, 1972) from early to late differentiation phase were construed to indicate a process of glyconeogenesis during differentiation in the tail of *Mabuya carinata*. The support for this suggestion comes from the studies, regarding the steady increase of glycogen from early to late differentiation phase (Chapter 3). Similar findings in *Hemidactylus flaviviridis* were also reported by Shah and Chakko (1967b) and Magon (1970) in *Hemidactylus flaviviridis*. A significant feature which has became apparent by the studies on the regenerating tails of both *Hemidactylus flaviviridis* and *Mabuya carinata* during the differentiation phase is the simultaneous synthesis of glycogen on one side and the utilization of lipid on the other. The above observations gain much support when viewed in the light of reported high activities of glycolytic enzymes studied by Ramachandran (1972) and Magon (1970) in *Mabuya carinata* and *Hemidactylus flaviviridis* respectively, such as aldolase, LDH and α-GPDH during the differentiation phase and highlights the fact that the glyconeogenetic process is an important subcellular biochemical adaptation of the regenerating reptilian tails. The high activity of malic enzyme reported by Ramachandran (1972) and Hiradhar (1972) in *Mabuya carinata* and *Hemidactylus flaviviridis* respectively, may be recalled at this juncture for the continuous replenishment of a pyruvate pool for oxaloacetate production, a part of which could be used for the continuation of the TCA cycle and another part for the formation of phosphoenol pyruvate for glyconeogenesis. This possibility of glyconeogenesis is accelerated by the levels of LDH, acetoacetate and acetyl Co A in animal tissues (Krebs *et al*., 1963). The reported high activity of LDH and MDH (Shah and Ramachandran, 1970) and BDH (Ramachandran, 1972) at this phase is self explanatory. Reported high activity of α-GPDH by Ramachandran (1972) during the differentiation phase reflected its role in channelization of the glycerol moities set free during the lipolysis, towards glyconeogenesis.

It is interesting to note that distribution pattern and significant fluctuations in the ascorbic acid (AA) content in *Mabuya carinata* (Chapter 8) and

Hemidactylus flaviviridis (Shah et al., 1971). In both the lizards, there were two peaks of activities of AA; one during the wound healing and the other during the differentiation phase, of the tail regeneration. Twofold increase of AA during the wound healing phase of the tail regeneration in Mabuya carinata and in Hemidactylus flaviviridis (Shah et al., 1971) is correlated with its possible role in the laying down of matrix material at the wound surfaces. Such an association of AA in the process of wound healing is reported by a number of investigators (Bourne, 1953; Zamanskii and Lopushanskii, 1955; Schauble et al., 1956).

It is interesting to note that ascorbic acid and ascorbic acid oxidase have both been considered to be an alternative to cytochrome oxidase as a terminal oxidase in plant and animal tissues (James, 1957; Goodwin, 1960; Mapson, 1953; Meiklejohn, 1953 and Chinoy, 1969a,b). As compared to other TCA cycle enzymes, the activities of cytochrome oxidase was found to be relatively less in the differentiating tissues (Ramachandran, 1972) and the reported five fold increase of AA (see Chapter 8 and Table 8.1) may be correlated in this light with its possible role in respiratory chain, by transforming into its free radical monodehydroascorbic acid, which is a more powerful electron donor than AA itself, thus playing an identical role as cytochrome oxidase (Goodwin, 1960; Mapson, 1953; Meiklejohn, 1953 and Chinoy, 1969a). It may be recalled here that Banerjee and Ganguli (1964) have suggested the influence of AA on hexokinase activity in glycogenesis. The presently observed parallel increase of both glycogen and AA during differentiation phase appear to be well in line with the above suggestion. It has become quite obvious that, though there is similarity in the activities of AA during corresponding phases of the tail regeneration in the two lizards, *viz*., *Mabuya carinata* and *Hemidactylus flaviviridis*, the concentration of AA in the former is considerable lower than that seen in the latter (Chapter 8). This could be indicating lower requirements of the vitamin in *Mabuya carinata* compared to that in *Hemidactylus flaviviridis* in the metabolic activities during tail regeneration. Further detailed studies on this aspects are necessary.

A histochemical study on the activities of the hydrolytic enzymes, acid phosphatase (Chapter 4) and alkaline phosphatase (Chapter 5) revealed one peak activity by the former and two by the latter in the regenerating tail of Mabuya carinata. The uniform distribution of alkaline phosphatase in the normal as well as in the regenerating tail tissues, as compared to acid phosphatase is indicative of its role in various biochemical reactions of the regenerative process. The presence of acid phosphatase in the epidermal region of the skin (Chapter 4) and an identical localization of RNA (Chapter 6) in the same region is correlated with synthesis of keratin, a feature met with the moulting skin; a similar function is proposed for alkaline phosphatase too. A complete absence of acid phosphatase in the normal tail muscles as well as in the developing muscles seems to be unique in both the lizards studied in this laboratory; contrast to the higher activity of alkaline phosphatase which is correlated with its possible role in the transport of phosphorylated metabolites. The high activity of acid phosphatase at the wound surface (Chapter 4) is correlated with phagocytosis and autolysis which are expected during the wound healing process. Identical localization of alkaline phosphatase in the normal as well as at the cut end of the muscles, during wound healing and preblastemic phases is correlated with its possible role in transport of phosphorylated metabolites (glycogen) as envisaged by Vallyathan and George (1965), (Chapter 3). An increased level of acid phosphatase during blastemic phase is correlated with the high levels of nucleic acids whereby the role of this enzyme in nucleic acid synthesis is discussed. An increased level of nucleic acids during the differentiation phase is also correlated with a parallel increase of alkaline phosphatase and G6PDH. Support for this comes from the reported suggestion of Rogers (1960) that alkaline phosphatase is concerned with phosphate transfer in DNA metabolism.

A study of cholinesterases in the normal and regenerating tissues of the tail revealed interesting results. Simpson (1964) and Singer (1955) and others have provided experimental data with regard to the influence of nerves and nerve cord on tail regeneration in lizard. In the present studies it has been noted that there is a negligible amount of AChE and BuChE during the earlier phases of tail regeneration (wound healing to blastema) which has prompted the suggestion that no neurotrophic agents are involved in the early phases of regeneration. The response of differentiating muscles towards AChE and BuChE indicates their possible role in histo-differentiation of muscles. The exact role of cholinesterases and the ependyma in the tail regeneration in lizards is yet to be properly understood. Experimental studies on the nerve supply in the normal and various phases of regeneration, might provide some clues regarding the exact role played by the nervous system in this fascinating process.

With the completion of the hectic proliferative and differentiative activities during the differentiation phase, the following growth phase is characterized by a gradual reduction in the contents of the metabolites such as glycogen and lipid, the structural ingradients viz., proteins, nucleic acids, and the vital factors such as vitamin C and the enzyme complements, in both the lizards studied in this laboratory, which marked the attainment of physiological and morphological maturity of the various tissues of the fully regenerated tail, as was in the original normal tail.

Finally, on the highlights of the various metabolic and biochemical reactions involved therein in the present investigations may be summarized as follows.

The tissues of the normal tail are chiefly adapted for anaerobic glycolysis and the early phases of regeneration, starting from wound healing to early differentiation are marked by a synthetic phase, for lipids, nucleic acids, proteins and a gradual acquisition of the necessary enzymological machinery for the utilization of lipid. The differentiation phase is characterized on one side by the active utilization of lipid for meeting the energy requirements of the highly active tissues and on the other side by the synthesis of glycogen through glyconeogenesis. Finally, along with the attainment of the fully regenerated condition which is histomorphologically, anatomically and physiologically, more or less similar to that of the normal tail, a biochemical restoration is also evidenced by its metabolites from carbohydrate to lipid and back to carbohydrate during the tail regeneration is characteristic of both the lizards studied in this laboratory.

Finally in a general comparison, both the type of lizards *viz.*, *Hemidactylus flaviviridis* and *Mabuya carinata* revealed more or less an identical pattern of distribution of metabolites, and the enzymes concerned, eventhough they belong to different families, Gekkonidae and Scincidae respectively.