INTRODUCTION

Standing out distinctly amongst the many marvels and wonders of the animal world is regeneration, the most fascinating and alluring phenomenon for biologists of the modern era. It is well established that amongst vertebrates, amphibians possess the highest regenerative capacities. Lens, retina, liver, eye, intestine, limbs and tail are all restored when lost by the amphibians especially the urodeles. In the case of amniotes only a few reptiles have retained the regenerative abilities that too, only of limbs and tails. The birds and mammals are known to exhibit only physiological regeneration.

As regenerative processes call~~s~~ for generation of additional units of structure and cellular organelles, synthesis of new molecules is obviously of paramount importance. Moreover, for the fulfilment of regeneration, apart from the synthesis of biochemical molecules and cells, it also calls for a multiplication and assemblage of the basic units so produced in the right way so as to build up the original histoarchitectural pattern of tissues. This orderly construction of histoarchitecture with materials produced indigenously

must indeed be controlled by a well knit controlling blueprint
wherein resides the blueprint
and directing system; the blue print of which resides where is something that remains unknown and speculative.

Many scientists all over the world are in pursuit of knowlege a knowhow of the various aspects of regeneration both of invertebrates as well as vertebrates. Most of the work on vertebrate tissues have been done on amphibian appendages. Some work has been extended to regeneration of reptilian tails though more of a morphological and histological nature. The physiological and biochemical aspects involved in this process were least explored. All the information available on this aspect is from experiments ~~angle are~~ on the regenerating amphibian appendages done mainly by Geczik and Wolsky (1959), Wolfe and Cohen (1963) and Schmidt and Weidman (1964) on the regenerating tail of adult Triturus, regenerating limb tissues of Triturus viridescens and on the regenerating limbs of Triturus viridescens and Diemictylus viridescens respectively. Though such works are carried out in different animals, especially in urodeles, a general lack of knowlege? is ~~felt~~ available in the regenerating reptilian tissues. Most of the work in the regenerating lacertilian tail are ~~of~~ morphological and histoanatomical in nature (Duges, 1829;

Hooker, 1912; Woodland, 1920). Other studies ~~on~~ along these lines are those of Quattrini (1954) on *Lacerta cicula*; Huges and New (1959) on *Sphaerodactylus*; and Moffat and Bellairs (1964) on *Lacerta vivipera*. Some of the biochemical and experimental works are those of Kamarin and Singer (1955) and Simpson (1964) who have reported on the influence of nerve cord and ependyma in the regenerat~~ing~~ion of the tail of *Anolis carolinensis* and *Lygosoma laterale* respectively. Licht (1967) studied the effect of certain hormones in the growth and tail regeneration in the lizard, *Anolis carolinensis* and Maderson and Licht (1968) reported the influence of temperature and nutrition on the regenerating tail of *Anolis carolinensis*. ~~The~~ Exploration on the biochemical aspects of regeneration has been ~~was very much~~ lacking in the reptilian tissues or do you mean systems

This vacuum in the study of reptilian tail regeneration was filled by Kamarin and Singer (1955), Simpson (1964) and Licht (1967) and by Shah and Chakko and Shah and Magon, from this laboratory. They studied the physiological and biochemical aspects of regeneration by a study of the distribution of various metabolites and enzymes in the regenerating tail of the house lizard, *Hemidactylus flaviviridis*.

It was in the wake of such limited information available on regenerating amniote tissues and as a follow up of the works conducted in this laboratory on the regenerating tail of *Hemidactylus flaviviridis* that it was found worthwhile to extend the studies to include work further and explore some of the metabolical aspects in the regenerating tail of another lizard *Mabuya carinata* so as not only to expand our horizon of knowledge on regeneration but also to understand the similarities and or dissimilarities involved therein between the regenerating tissues of amphibia and reptilia and even amongst the reptiles ~~itself~~ themselves. Histochemical distribution of dehydrogenases in the regenerating tail of *Mabuya carinata* constitute the most dominant feature of the present study and attempts will be made to compare, contrast and correlate the findings hatched out herein with those of a similar nature evolved ? out in this laboratory on the basis of studies conducted on *Hemidactylus flaviviridis* and also on *Mabuya carinata* chiefly to explore the distribution of metabolites and hydrolytic enzymes.

did you really hatch them?

Since carbohydrates and lipids serve as the energy yielding sources for living tissues, it is rather pertinent to study some of the emzymes that play important roles in the catabolism and anabolism of these metabolites. Aldolase and lactate dehydrogenase are the important pivotal enzymes of the glycolytic pathway. The Presence of these two enzymes could be taken as an index of glycolytic activity. and ~~It~~ it was with this view in mind that a histochemical study of these two enzymes in the normal and regenerating tail of Mabuya carinata was undertaken.

Aldolase, the important enzyme of the glycolytic pathway, ~~is~~ was studied histochemically. Aldolase ~~is~~ was found to be present in adequate~~ly~~ amounts in all phases of regeneration with an increased activity above the normal level during the progressive phases of regeneration. This steady activity of aldolase with no fall below the normal level clearly indicates the existence of anaerobic energy yielding reactions through the Embden Meyerhoff pathway either by the breakdown of glycogen stored up in the tissues or glucose supplied by the circulating blood.

Lactate dehydrogenase (LDH), another important enzyme of the glycolytic pathway marking the terminal part of anaerobic glycolysis is found to have a slightly different distribution. This enzyme is found to register a fall below the normal level during the early phases of regeneration followed by a highly pronounced activity during the progressive phases of regeneration, finally attaining the normal level in the fully regenerated tail by a gradual decrease of activity during the growth phase. An identical pattern of distribution of malate dehydrogenase (MDH) one of the enzymes of TCA cycle establishes a close correlation between the activities of these two enzymes. This correlation highlights the existance of a pyruvate centered short cycle during the tail regeneration of Mabuya carinata, closely linked with lactate, malate and oxaloacetate and very much comparable to the pyruvate carboxylase shuttle present in the vertebrate liver and muscle. This cycle has the advantage of supplying $NADPH_2$ an important cofactor for lipogenesis. Further significancesare correlated with the cycle.

To confirm conclusively the existance of the pyruvate centered cycle and also to study the role of the HMP shunt pathway during the tail regeneration in Mabuya carinata, the distribution of malic enzyme and glucose-6-phosphate dehydrogenase ~~is~~ was undertaken. The distribution of malic enzyme parallel to those of lactate and malate dehydrogenases gave the added evidence for the existence of a pyruvate centered cycle. Glucose-6-phosphate dehydrogenase (G6PDH) activity tended to be high during the blastema and differentiation phases of regeneration proving not only the significance of the shunt pathway as an alternative pathway of glucose utilization during these phases but also in the yielding of reduced coenzyme II which is useful for lipogenesis.

The higher content of lipids during blastema and early differentiation phases noted by other investigators in this field are in good correlation with the distribution of the above enzymes. It was also noted that the lipid content dwindled during the differentiation phase, proving the importance of lipids as the main source of energy for the actively proliferating cells. To understand the mode of lipid utilization a study of β-hydroxy butyrate dehydrogenase (BDH) and α-glycerophosphate dehydrogenase (α-GPDH) ~~is~~ was carried out. BDH ~~is~~ was found to be highly active only during the blastema and differentiation phases indicating its importance in fatty acid breakdown so as to facilitate their entry in the TCA cycle. ~~Whereas~~ αGPDH ~~is~~ was noted to be more active during all phases except during the late blastema and differentiation phases, whence it tended to be slightly higher. This leads to the conclusion that due to its strategic position in the metabolical map, it could be playing a dual role during regeneration in Mabuya carinata by participating in the reversible reaction between glycerol and the glycolytic pathway as per need of glycerol anabolism or catabolism during different stages of regeneration.

Earlier workers (Wolfe and Cohen, 1963) and (Schmidt and Weidman, 1964) in the field of regeneration have attributed a negligible role for the TCA cycle during the phenomenon of regeneration? But at the same time the gradual depletion of lipids during the differentiation phase is also evidenced. This anamoly and the total neglect on the study of isocitrate dehydrogenase (ICDH) prompted the present study on succinate dehydrogenase (SDH) and ICDH. These two enzymes are found to be almost negligible in concentration and in distribution in the normal tail tissues and also during the earlier phases of regeneration. But appreciably adequate activities are noticed during late blastema and differentiation phases showing the acquisition of these enzymes by the cells to effectively oxidize lipids through the TCA cycle so as to cope with the extra energy requirements of actively dividing and differentiating cells. The significance of ICDH activity slightly higher than that of SDH can be understood when the products of ICDH catalyzation are taken into consideration.

The product, α-ketoglutarate is an important starting point for the synthesis? production of a number of amino acids. Further, ICDH together with the malic enzyme seems to play the role of satellite enzymes to the HMP shunt in the production of $NADPH_2$.

Finally the distribution of cytochrome oxidase and diaphorases ~~are~~ was studied histochemically to ascertain the validity of the concepts derived above herefrom. Cytochrome oxidase activity could establish the presence of an intracellular electron transport system during regeneration coupled to dehydrogenase activities ~~to~~ with the ultimate yield of high energy phosphate bonds. The NAD and NADP diaphorases followed a parallel distribution pattern in comparison to their corresponding dehydrogenase i.e., the NAD dependent and NADP dependent dehydrogenases. This conclusively proves that there is a continuous oxidation of reduced coenzymes whereby the steady replenishment of coenzymes is ensured for the various dehydrogenases at work during regeneration.

The present investigations are in line with those of earlier workers ones of this nature conducted on the tail of <u>Hemidactylus flaviviridis</u> from this laboratory. Apart from giving an insight into the possible metabolic patterns and adaptations in the regenerating reptilian tail, it is also hoped that the present studies may also open up new avenues and vistas for further fruitful research helpful in the identification and elucidation of factors of wider implications in the most alluring and unyielding physiological phenomenon that has ever confronted the scientific world.