CHAPTER 1

ALDOLASE IN THE NORMAL AND REGENERATING TAIL OF THE SCINCID LIZARD, MABUYA CARINATA: A HISTOENZYMOLOGICAL STUDY

Aldolase catalyzes one of the key steps in the glycolytic cycle converting fructose-1,6-diphosphate into dihydroxy acetone and glyceraldehyde-3-phosphate; two isomers interchangeable by the mediation of triose phosphate isomerase. The presence of this enzyme in any animal tissue can conclusively establish the existance of glycolytic activity wherein there is a breakdown of glycogen or glucose to pyruvate anaerobically. Aldolase is found in all glycolyzing cells and has been particularly studied in striated muscle (Long, 1961). Roodyn (1956, 1959) has reported the association of this enzyme with the nuclei of liver cells. It is also found abundantly in the supernatant fraction of liver and other tissue homogenates (Duve et al., 1962).

Schmidt (1960) and Chakko (1967) have histochemically studied the distribution of glycogen in the normal and regenerating limb tissues of Triturus viridescens and normal and regenerating tail tissues of Hemidactylus flaviviridis respectively. With reference to

glycogen distribution studies, Chakko (1967) has suggested different levels of glycogen utilization during various phases of tail regeneration. Needham (1952), Fredrickson and Gordon (1958), Rossiter and Strickland (1960), Schmidt (1962, 1966a & b), Chakko (1967) and Shah and Chakko (1967) have all reported noticeable fluctuations in the amount of utilization of metabolites and the concentration of the enzymes concerned in different tissues including the regenerating ones of amphibia and reptilia. The constancy and or fluctuations in the utilization of glycogen through glycolysis during different phases of regeneration may more or less be ascertained by a study of the distribution of a key enzyme like aldolase. The only two instances of aldolase demonstration in the regenerating tissues to date are by Schmidt and Weidman (1964) and Magon (1970). The scarce literature available about aldolase during regeneration and the reports of fluctuations in the utilization of metabolites mentioned above have prompted the present study in the normal and regenerating tail of the Scincid lizard, Mabuya carinata.

MATERIALS AND METHODS

The adult Mabuya were selected and the tails autotomized by pinching off the tail one to two inches distal to the vent. The normal and various stages of the regenerating tail after autotomy were blotted to remove blood and other tissue fluids and were immediately fixed on a chuck of a cryostat microtome maintained at -20°C. Longitudinal and transverse sections of 10-15 μ thickness were cut and incubated at room temperature for about 30 minutes in the incubation medium consisting of the following ingredients.

Sodium fructose-1,6-diphosphate (substrate) 0.02 M	2 ml
Nicotinamide adenine dinucleotide 1 mg/ml	1 ml
Nitro-BT 1 mg/ml	1 ml
Arsenate-HCl buffer, 0.05 M, pH 7.6	2 ml

A few sections treated in water at 80°C before incubation and a few incubated in a substrate blank medium served as the controls.

OBSERVATIONS

NORMAL TAIL (Fig.1)

Enzyme activity worth noticeable in the skin was

only in the cells of the stratum germinativum with no activity in the dermis and beta cells. The alpha cells exhibited a faint colouration more due to their close approximation with the stratum germinativum.

The skeletal muscles represented the highest epitome of enzyme activity in the normal tail and the localization was both sarcoplasmic and mitochondrial. The peripheral smaller fibres showed more prominent activity than the inner bigger fibres in all the muscle fasciculi.

The cartilage cells of the centrum showed noticeable activity with no activity in the osteocytes or matrix of the vertebrae. In the nerve cord, there was a poor to nil activity in the grey matter and white matter respectively.

REGENERATING TAIL

Wound healing phase: (4-5 days after autotomy) (Figs.2 & 3)

The wound epithelium in contrast to the underlying subapical cells showed an appreciable aldolase activity.

Preblastemic and blastemic phases: (10-12 days after autotomy) (Figs.4 & 5)

The stratified wound epithelium, now the blastemic

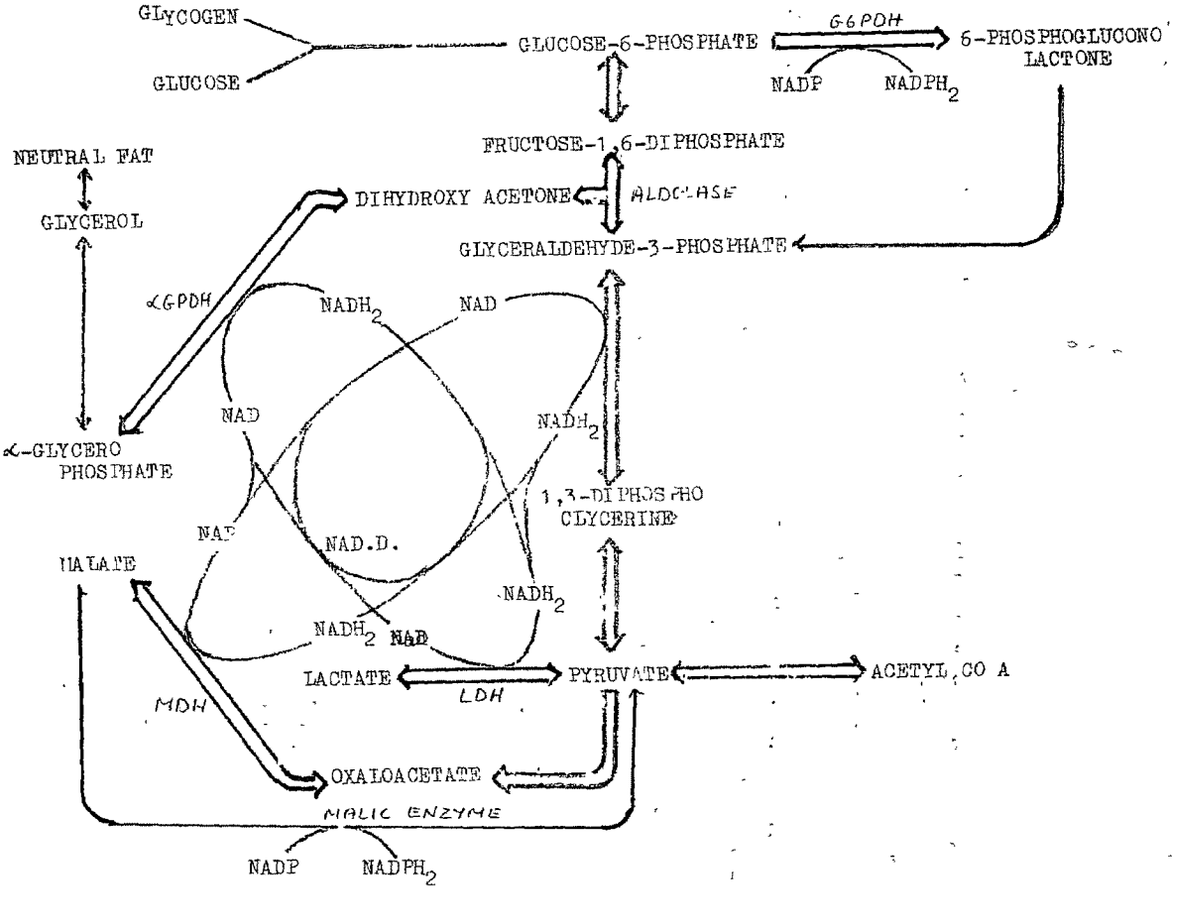
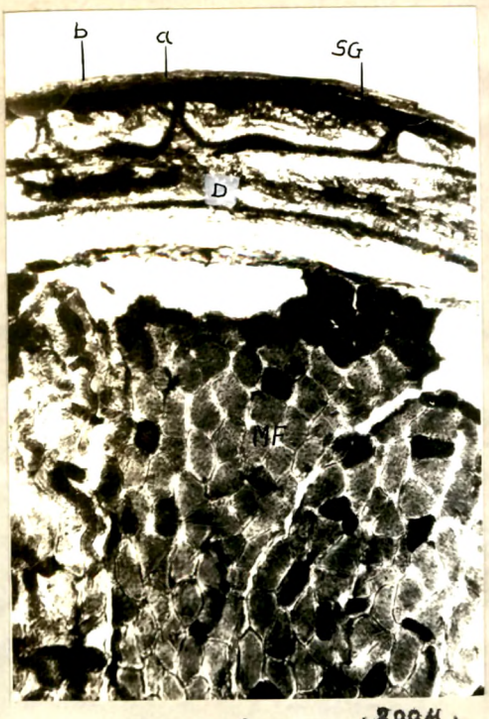

GLYCOGEN
GLUCOSE
GLUCOSE-6-PHOSPHATE
G6PDH
6-PHOSPHOGLUCONO LACTONE
NADP
NADPH2
FRUCTOSE-1,6-DIPHOSPHATE
NEUTRAL FAT
GLYCEROL
DIHYDROXY ACETONE
ALDOLASE
GLYCERALDEHYDE-3-PHOSPHATE
αGPDH
NADH2
NAD
α-GLYCERO PHOSPHATE
1,3-DIPHOSPHO GLYCERINE
NAD.D.
MALATE
LACTATE
LDH
PYRUVATE
ACETYL CO A
MDH
OXALOACETATE
MALIC ENZYME
NADP
NADPH2

FIG. 1



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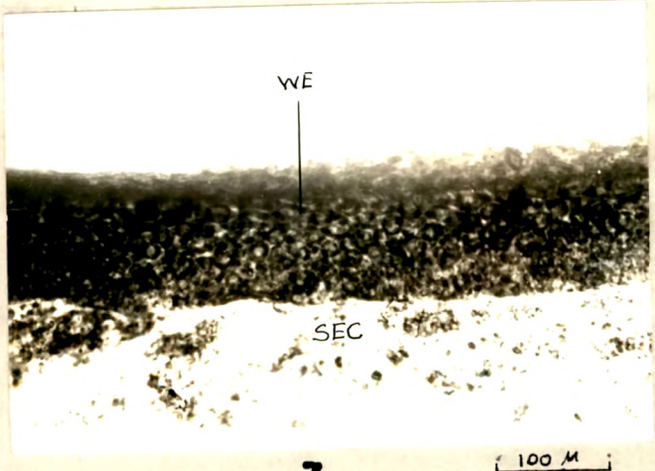

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EXPLANATIONS FOR FIGURES

Fig. 1. Photomicrograph of T.S. of normal tail showing aldolase activity in the stratum germinativum and muscles.

Fig. 2. Photmicrograph of wound healing phase. Note the enzyme activity in the wound epithelium.

Fig. 3. Photomicrograph of wound epithelium showing aldolase activity.

ABBREVIATIONS

a	-	alpha cells
b	-	beta cells
CE	-	Cut end of tail
D	-	Dermis
MF	-	Muscle fibres
SC	-	Scute
SCA	-	Scab
SEC	-	Subepithelial cells
SG	-	Stratum germinativum
WE	-	Wound epithelium

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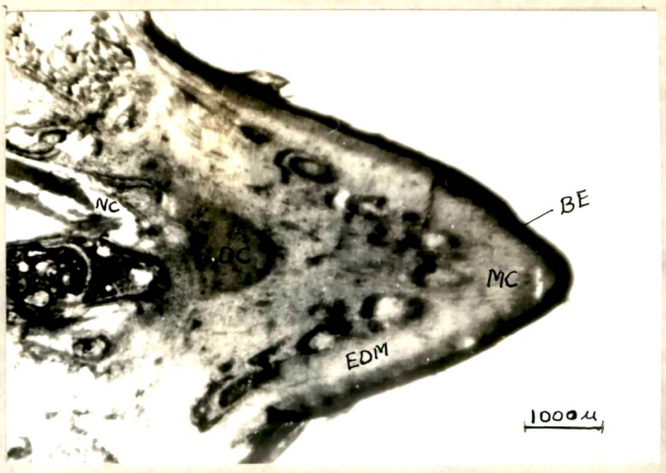

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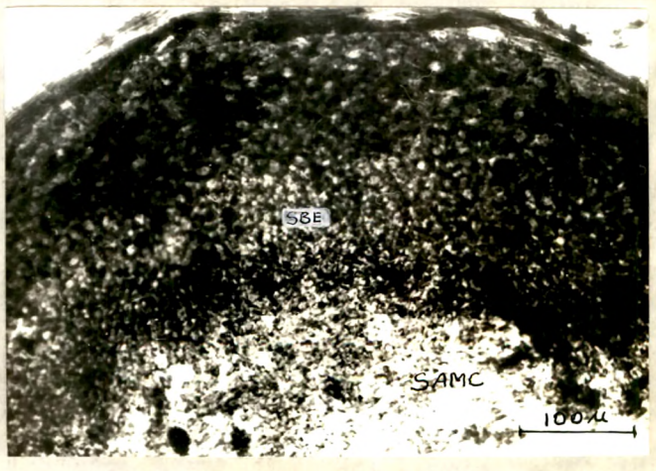

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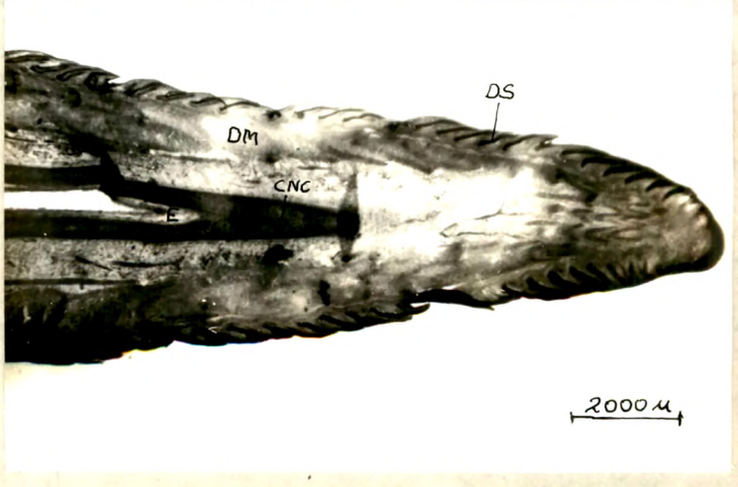

Fig. 4. Photomicrograph of L.S. of blastema depicting aldolase activity. Note the enzyme activity in both the blastemic epithelium as well as the mesenchymal cells.

Fig. 5. Blastemic epithelium showing pronounced enzyme activity.

Fig. 6. Photomicrograph of L.S. of differentiating tail showing aldolase activity in the various differentiating structures.

ABBREVIATIONS

BE	-	Blastemic epithelium
CNC	-	Cartilaginous neural canal
DC	-	Differentiating cartilage cells
DM	-	Differentiating muscles
DS	-	Differentiating scales
E	-	Ependyma
EDM	-	Early differentiating muscles
MC	-	Mesenchymal cells
NC	-	Nerve cord
SAMC	-	Subapical mesenchymal cells
SBE	-	Stratified blastemic epithelium

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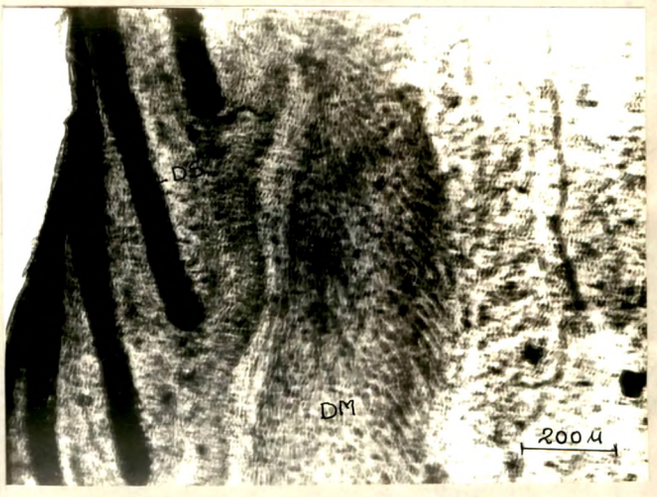

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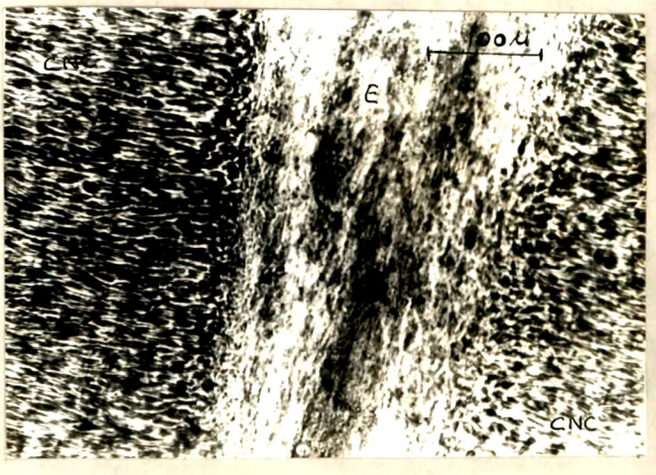


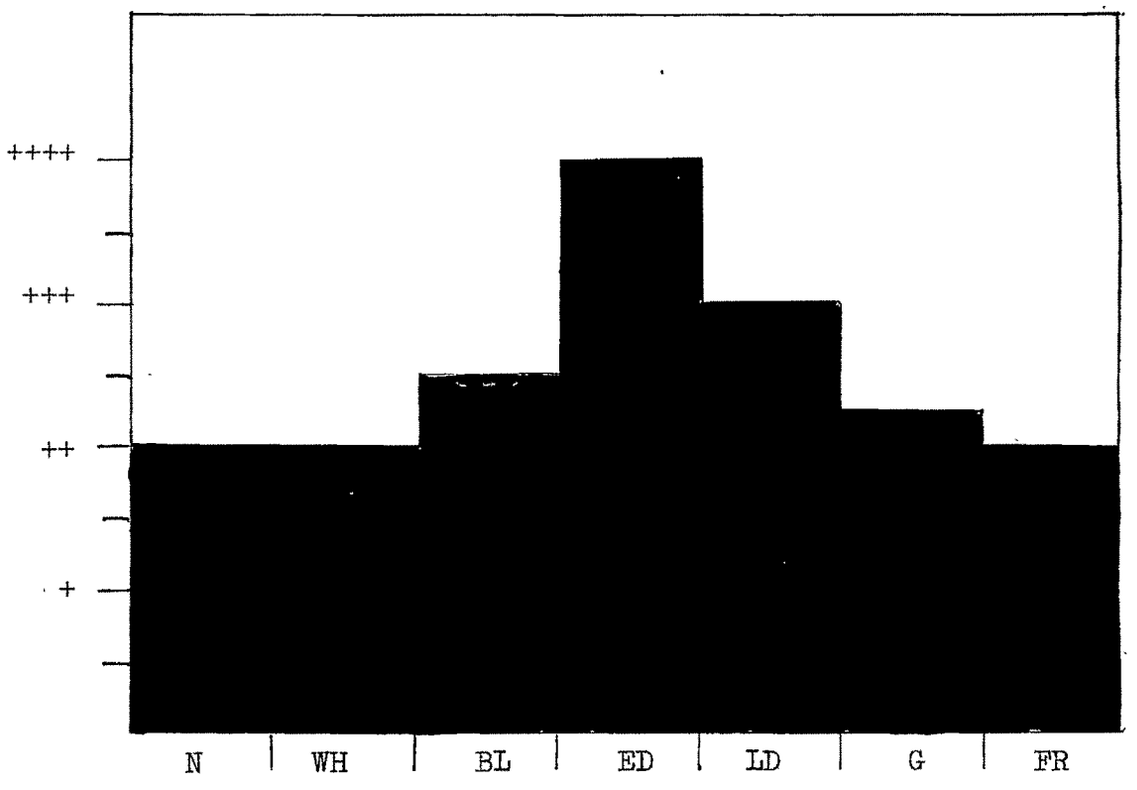
Fig. 7. Photomicrograph showing aldolase activity in the differentiating scales and muscles.

Fig. 8. Photomicrograph of cartilaginous neural canal with ependyma demonstrating aldolase activity.

ABBREVIATIONS

CNC	-	Cartilaginous neural canal
DM	-	Differentiating muscles
DS	-	Differentiating scales
E	-	Ependyma

Graphic representation of the changes in the aldolase distribution pattern during the various stages of tail regeneration



N; Normal ; WH: Wound healing phase;
BL: Blastemic phase; ED: Early differentiation phase;
LD: Late differentiation phase; G: Growth phase;
FR: Fully regenerated tail.

epithelium showed highly enhanced aldolase activity when compared with than the wound epithelium. The mesenchymal cells forming the bulk of the blastema also showed a moderately high aldolase activity. The cut end of the original tail tissues exhibited a pronounced enzyme reaction.

Differentiation phase: (starting 14 days after autotomy) (Figs.6-8)

The stratified epithelial cells of the regenerate of the early differentiation phase showed a high concentration of aldolase which seemed to diminish with the onset of cellular differentiation of skin and development of scales. Ultimately with the complete differentiation into epidermis and dermis, the stratum germinativum stood as the lone entity equipped with remained as the only tissue showing any aldolase activity. As the myogenesis and chondrogenesis progressed the differentiating myofibres and chondrocytes showed increasing levels of aldolase concentration finally attaining the highest level during the late differentiation phase. During early differentiation, the mononuclear myoblasts and myocytes displayed only cytoplasmic localization of the enzyme whereas during late differentiation the multinucleated myofibres also acquired the mitochondrial localization. The differentiating chondrocytes of the neural tube showed appreciably high activity of aldolase. The cells of the ependyma also

showed though poor but noticeable enzyme localization. On the whole, the various cells of the regenerate at this phase taken together was seen to possess an enzyme complement much above that of the cells of the normal tail.

Growth phase: (starting 30 days after autotomy)

During this phase there was a gradual but continuous fall in the aldolase activity with the result, in the fully grown condition with the attainment of morphological and functional maturity the various cells of the fully grown regenerate showed an enzyme distribution pattern and localization similar to that of the corresponding cells of the normal tail. In the skin, as in the normal one, only stratum germinativum represented any enzyme activity. In the muscle fibres, the peripheral ones showed greater activity than the inner ones. The fully differentiated chondrocytes of the neural canal showed a much reduced activity as compared to the differentiating ones, while there was a sustained poor enzyme activity in the ependyma of the fully grown regenerate.

DISCUSSION

Aldolase as an indicator of glycolytic metabolism

is well established by studies on various vertebrate tissues (George and Talesara, 1961, in pigeon muscle; Long, 1961, in striated muscle; Roodyn, 1956, 1959, in the nuclei of liver cells; and Duve et al., 1962, in liver and other tissue homogenates). In the present study, the normal tail tissues of Mabuya carinata have shown a positive response for aldolase activity. The enzyme was noticeable more in the stratum germinativum of the epidermis and in the muscle fibres. These two tissues thus seem to represent the most important sites of glycolytic activity in the normal tail. The distribution pattern of this enzyme noticed in the normal tail tissues seems to be in conformity with the findings of Schmidt and Weidman (1964) in the limb of adult newt, Diemictylus viridescens and Magon (1970) in the tail of the house lizard, Hemidactylus flaviviridis. The presence and role of aldolase in the skin and muscles of the normal tail are further confirmed by the amount of glycogen demonstrated in these tissues in the tail of Mabuya carinata (Shah and Radhakrishnan, unpublished); in the limb of Diemictylus viridescens (Schmidt, 1962) and in the tail of Hemidactylus flaviviridis (Shah and Chakko, 1967). The presence of both glycogen and aldolase in the stratum germinativum

is indicative of the utility of glycolysis as an important source of energy for the continuous formation of new generations of epidermal cells and also for the development of new scales. It is interesting to note in this connection the suggestion of Shah and Chakko (1967) that the glycogen and phosphorylase activity in the beta and alpha cells of the new generation (formed before ecdysis) provide necessary material for the synthesis of keratin in the skin of the lizard tail. Further evidences in favour of glycolytic dependence by the cells of the skin come from reports of high glycogen content and phosphorylase activity (Bradfield, 1951; Kasabyan, 1956; Berlin, 1958, 1959) in the human epidermis. Moreover as these workers have suggested, the anaerobic glycolysis seems to be an important feature of the regenerating epithelial cells. The reports of Montagna and Ellis (1958) and Shah and Chakko (1967) stating the ability of epidermal cells to synthesize glycogen strengthen the above observation in the tail of Mabuya carinata. The high incidence of aldolase noticed in the present study may thus be a definite indication of continuous catabolism and anabolism of glycogen in the skin. The distribution pattern of aldolase denoted three types of muscle fibres with respect to their intensity of

enzyme activity. Similar observation has been made by Magon (1970) in the tail of Hemidactylus flaviviridis. The glycogen content and phosphorylase activity were also found to follow a similar pattern in the tail muscles of Mabuya carinata (Shah and Radhakrishnan, unpublished) and Hemidactylus flaviviridis (Shah and Chakko, 1967). The high aldolase active fibres seem to be the glycogen loaded quick contracting variety (George and Talesara, 1961). On the whole, the normal tail of Mabuya carinata seems to be quite dependant on the glycolytic pathway for its subservience.

A predominant carbohydrate metabolism was evidenced in the wound epithelium (in the present study) by the observation of high aldolase activity and seems to be in accordance with the distribution of aldolase and glycogen noticed in the wound epithelium of lizard tails (Magon, 1970; Shah and Radhakrishnan, unpublished; Shah and Chakko, 1967) and amphibian limb (Schmidt and Weidman, 1964 and Schmidt, 1962).

During the blastemic phase, the enzyme activity was found to be poor both in Diemictylus viridescens (Schmidt and Weidman, 1964) and Hemidactylus flaviviridis (Magon, 1970). Contrary to the above observations, the cells of the regeneration blastema of Mabuya carinata

showed a moderately high aldolase activity and this is suggestive of a persistant glycolytic activity during this phase as well. The low level of glycogen and phosphorylase reported by Schmidt (1968) and Shah and Chakko (1967) in Diemictylus viridescens and Hemidactylus flaviviridis taken together with the present observation of aldolase activity in the blastema is indicative of an alternate carbohydrate source not necessarily a polysaccharide such as glycogen but perhaps a monosaccharide, such as glucose which can be readily is readily available availed of from the circulating blood. The accumulation of lactate in the regeneration blastema (Okuneff, 1933) and the high incidence of lactate dehydrogenase (Chapter 2) may be reflected in the aldolase activity reported above and could be drawn upon in support of the contention that the blastemal cells also depend upon glycolysis for their energy source.

With the onset of differentiation a gradual increase of aldolase activity in all the tissues of the regenerate was observed with the highest activity being attained at the late differentiation phase. The dividing and differentiating cells of the epidermis, myocytes and myofibres and to a certain extent the chondrocytes all registered increasing aldolase activity expressing

the enhanced glycolytic metabolism to satisfy the energy needs at this phase. This observation is in conformity with that of Magon (1970) and can be readily correlated with the progressive increase of glycogen and phosphorylase in this phase reported in the regenerating tail of the house lizard by Shah and Chakko (1967) and in the tail of Mabuya carinata (Shah and Radhakrishnan, unpublished). The increased activity of aldolase during the differentiation phase could also denote a high incidence of glyconeogenesis corresponding to the lipid utilization.

The growth phase was marked by a gradual decrease of aldolase activity denoting the attainment of structural and functional maturity by the differentiating tissues and cells, forming the fully regenerate tail and finally displaying the original pattern of distribution and localization characteristic of the normal tail.