CHAPTER 2

LACTATE AND MALATE DEHYDROGENASES (LDH & MDH) IN THE NORMAL AND REGENERATING TAIL OF THE SCINCID LIZARD, MABUYA CARINATA

Since metabolism plays an important role in the subservience and maintenance of cells, and the process of regeneration calls for ~~much~~ many cellular changes and reorganization, it is of most some importance to investigate some of the biochemical aspects involved therein. Studies on the activities of enzymes which usually give a clue to the metabolic state in the cells, have been done in the regenerating appendages of vertebrates, mostly in the amphibians, by some workers in recent times. Investigations on the histochemical localization and distribution of dehydrogenases such as lactate, malate, glucose-6-phosphate, α-glycerophosphate, succinate and isocitrate which catalyze important biochemical reactions in the various metabolic pathways in the regenerating tissues of vertebrates, have given a good insights into the pattern of metabolic changes that take place during different phases of regeneration. Geczik and Wolsky (1959) determined lactate, malate and succinate dehydrogenases in the

regenerating tail of adult Triturus. Wolfe and Cohen (1963) studied lactate, malate, glucose-6-phosphate, isocitrate and succinate dehydrogenases in the regenerating limb tissues of Triturus viridescens. Schmidt and Weidman (1964) investigated the histochemical distribution of all dehydrogenases mentioned above in the regenerating limb of the newt, Diemictylus viridescens. These studies revealed a higher activity of LDH and MDH and negligible activity of succinate and isocitrate dehydrogenases (SDH and ICDH) in the regenerating tissues. Based on these observations Schmidt (1964) has suggested a negligible role for the tricarboxylic acid (TCA) cycle with a strong pyruvate centered metabolism. He further postulated the possibility of an abbreviated TCA cycle through glyoxalate. From these, it is assumed, that glycolysis, the hexose monophosphate shunt (HMP) pathway and the abbreviated TCA cycle are the main energy yielding reactions at play in the various phases of regenerating amphibian appendages. Such investigations have not been undertaken to farther the insight into the phenomenon in reptiles. Only a meagre information from earlier workers in this laboratory who have studied the distribution of some important metabolites,

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hydrolytic enzymes and dehydrogenases in the regenerating tail of the house lizard, Hemidactylus flaviviridis is available. Barring this, information regarding such studies in the reptilian appendages are scanty. So the present study on the distribution of LDH and MDH in the regenerating tail of Mabuya carinata (a scincid lizard) was undertaken with a view to understand the similarities and or dissimilarities involved therein in the metabolism of regenerating reptilian tissues as compared with that of the regenerating amphibian tissues.

MATERIALS AND METHODS

The selected adult Mabuyas were maintained in the laboratory on a diet of young cockroaches. The autotomy of the tails was carried out by pinching off the tails about one to two inches distal to the vent. The normal and regenerating tails were autotomized, blotted to remove blood and tissue fluids and were immediately fixed on a microtome chuck in a cryostat maintained at -20°C. Longitudinal and transverse sections of 12-18 μ thickness were cut and incubated at room temperature for about 40 minutes in the media

adjusted to pH 6.5 to 7. The respective incubation media were prepared as given below.

Ingredients	LDH	MDH
Sodium lactate (substrate) 1 M	0.1 ml	-
Sodium malate (substrate) 1 M	-	0.1 ml
Nicotinamide adenine dinucleotide 0.1 M	0.1 ml	0.1 ml
Sodium cyanide 0.1 M	0.1 ml	0.1 ml
Magnesium chloride 0.05 M	0.1 ml	0.1 ml
Phosphate buffer 0.06 M , pH 6.8-7	0.25 ml	0.25 ml
Nitro-BT 1 mg/ml	0.25 ml	0.25 ml
Distilled water	0.1 ml	0.1 ml
Polyvinyl pyrrolidone	75 mg	75 mg

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

OBSERVATIONS

NORMAL TAIL (Figs.1, 2 & 1A, 2A)

Skin:

The outer beta and alpha layers of cells of the epidermis showed little or no localization of either LDH or MDH activity. However, a few of the inner layers

of alpha cells towards the stratum germinativum showed slight activity, representing the waning activity of the enzymes in these cells as they ~~are getting~~ become keratinized. Of all the layers of the epidermis, the cells of the stratum germinativum exhibited the maximum enzyme activity. The scutogenic cells also exhibited appreciable enzyme activity. In the dermis, the intercellular matrix, the scutes and the connective tissue lying above the muscle fasciculi, the localization of these enzymes was very poor. level of enzyme activity was poor ?

Muscle:

Of all the tissues of the normal tail, muscles depicted the highest concentration of enzymes, the localization of which was found to be both sarcoplasmic as well as mitochondrial. The mitochondrial localization was predominant as compared to the diffuse sarcoplasmic distribution. Though all the muscle fibres in each fasciculus were more or less of the same size, three types of fibres could be differentiated with regard to their mitochondrial number and enzyme concentration. All the peripheral fibres in a fasciculus were comparatively smaller in size with high enzyme intensity and mitochondrial number. Of the

remaining fibres, which were almost of the same size, some revealed fewer mitochondrial number and lesser enzyme activity, whereas others were intermediate to the above two types in enzyme activity and mitochondrial concentration.

Submuscular adipose tissue:

The submuscular adipose tissue also recorded adequate response towards LDH and MDH. The activity was seen more in the peripheral cytoplasm whereas the fat globules attained a purplish to blue coloration probably due to the tetrazoleum adsorption as envisaged by Schmidt (1963).

Vertebral column and Nerve cord:

The bone matrix and the cells of the vertebral column failed to show any enzyme response whereas the cartilage cells at the articulating ends of the centrum showed appreciable enzyme activity. Both LDH and MDH were also noticed in the grey matter of the nerve cord while a relatively weak response was exhibited by the white matter.

REGENERATING TAIL

Wound healing phase: (Figs.3 & 3A, 4A)

An Appreciable high enzyme activity could be seen

in the wound epithelium covering the cut end of the tail. The muscles and the nerve cord of the original tail stump subjacent to the cut end also showed high enzyme activity.

Preblastemic and blastemic phases: (Figs.4-6 & 5A,6A)

A remarkable increase of enzyme activity in the blastemic epithelium could be noticed during these phases. Parallel increase of these enzymes was noticed with the degree of stratification of the epithelium. The mesenchymal cells filling up the blastemal core showed a low enzyme activity as compared to the original tail tissues at the cut end from which they were derived as a result of dedifferentiation. Although all the mesenchymal cells showed a low enzyme activity a noticeably higher activity was seen in those cells underlying the blastemal cap.

Differentiation phase: (Figs.7-9 & 7A-9A)

During differentiation, in addition to the apical mesenchymal cells underlying the epidermis, the extending ependymal cells into the basal part of blastema, the cells differentiating into cartilagenous neural canal and the muscle fibres presented a relatively higher enzyme content. As the differentiation

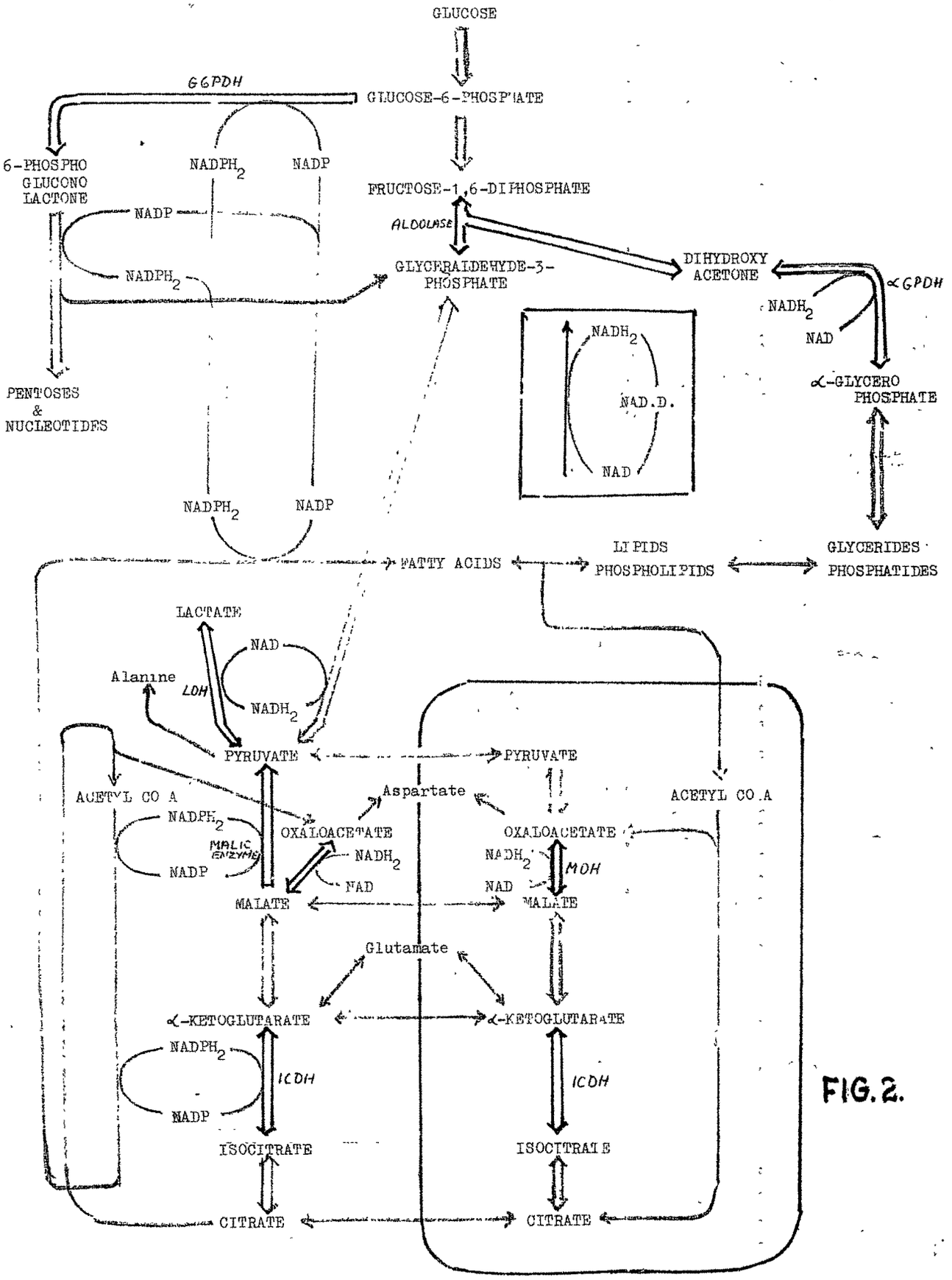
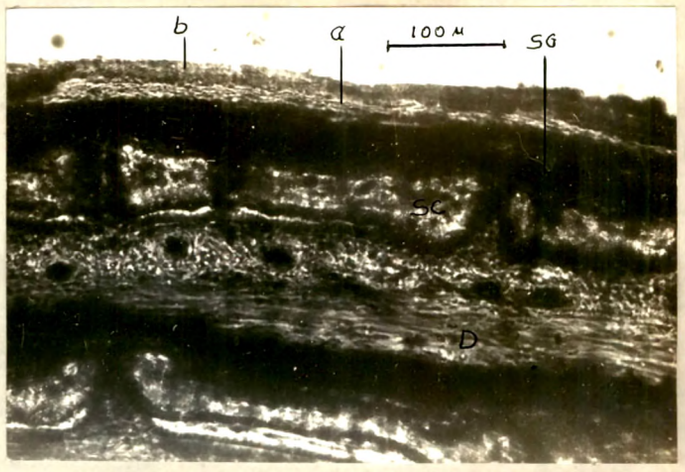

GLUCOSE
G6PDH
GLUCOSE-6-PHOSPHATE
6-PHOSPHO GLUCONO LACTONE
NADPH2
NADP
FRUCTOSE-1,6-DIPHOSPHATE
ALDOLASE
GLYCERALDEHYDE-3-PHOSPHATE
DIHYDROXY ACETONE
αGPDH
NADH2
NAD
α-GLYCERO PHOSPHATE
PENTOSES & NUCLEOTIDES
NAD.D.
FATTY ACIDS
LIPIDS PHOSPHOLIPIDS
GLYCERIDES PHOSPHATIDES
LACTATE
Alanine
LDH
PYRUVATE
ACETYL CO A
Aspartate
OXALOACETATE
MALIC ENZYME
MDH
MALATE
Glutamate
α-KETOGLUTARATE
ICDH
ISOCITRATE
CITRATE

FIG. 2.

1



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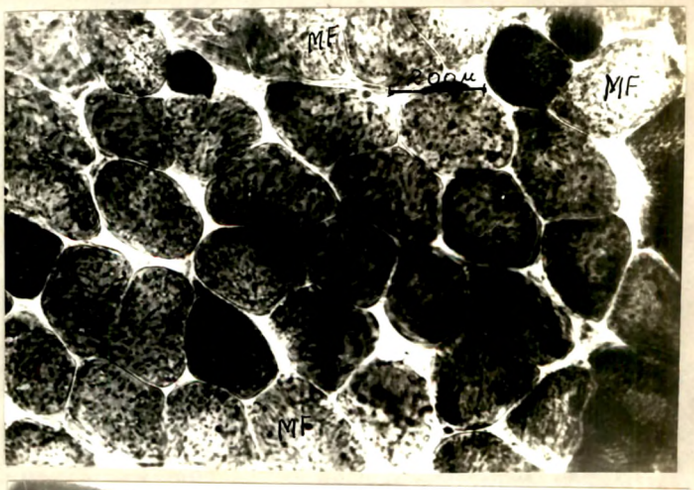

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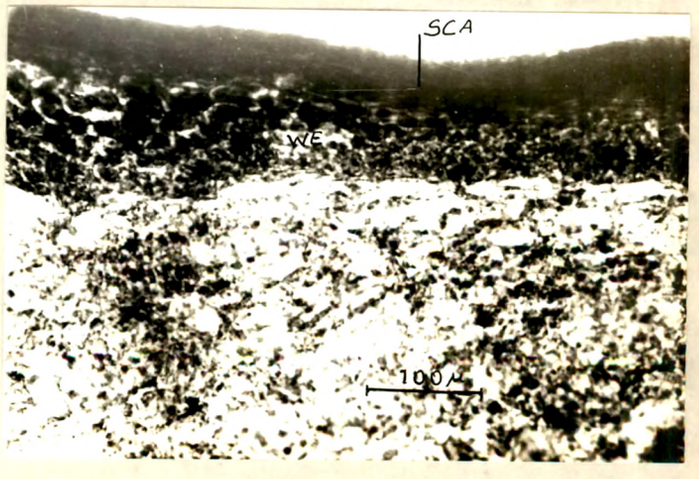


EXPLANATIONS FOR FIGURES

Fig. 1. Photomicrograph of T.S. of normal tail skin showing LDH activity. Note the intense enzyme activity in the stratum germinativum.

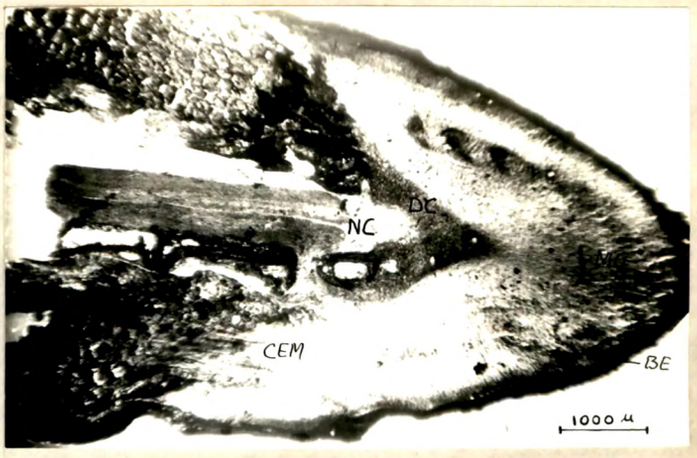
Fig. 2. T.S. of normal tail muscles depicting LDH activity.

Fig. 3. Photomicrograph of wound epithelium showing high LDH activity. Note the enzyme activity in the subepithelial cells as well.

ABBREVIATIONS

a	-	alpha cells
b	-	beta cells
D	-	Dermis
MF	-	Muscle fibres
SC	-	Scute
SCA	-	Scab
SEC	-	Subepithelial cells
SG	-	Straum germinativum
WE	-	Wound epithelium

4



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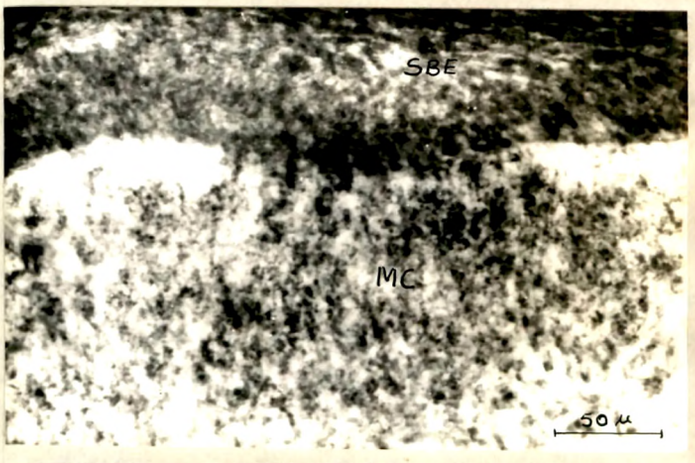

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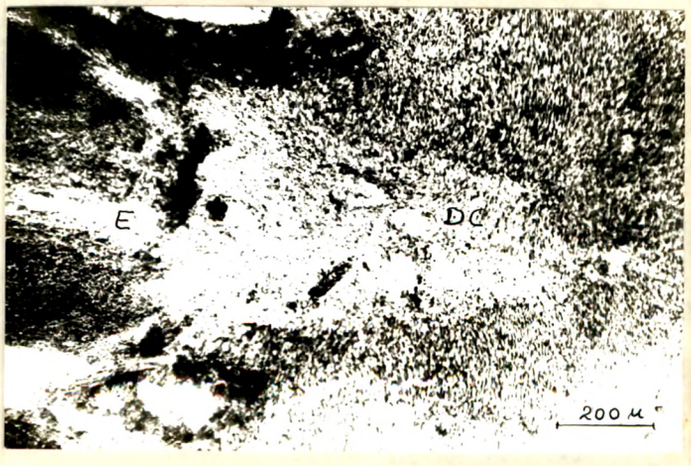


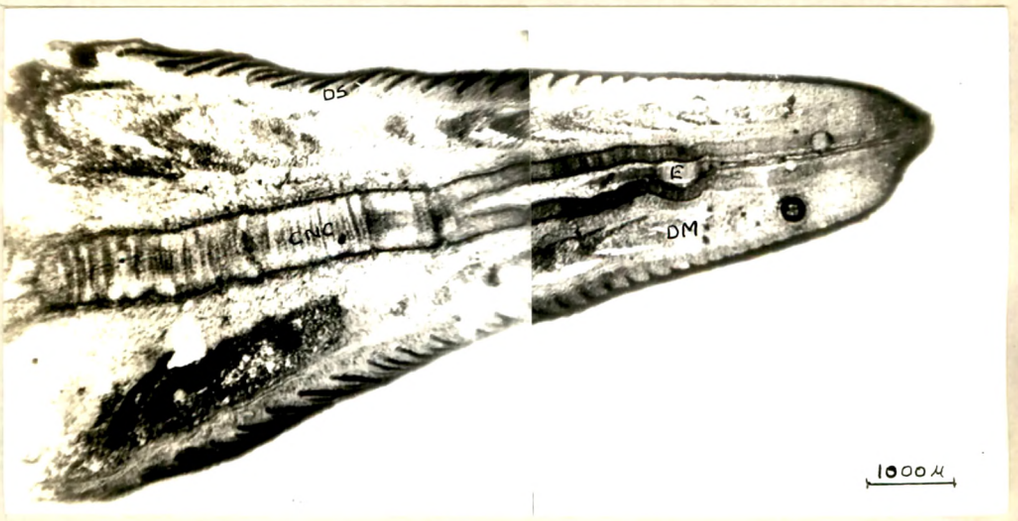
Fig. 4. Photomicrograph of L.S. of blastema showing LDH activity.

Fig. 5. Photomicrograph of blastemic epithelium with high LDH activity. Note the enzyme activity in the mesenchymal cells.

Fig. 6. Photomicrograph of early differentiating cartilage cells of the neural canal showing enzyme activity.

ABBREVIATIONS

BE	-	Blastemic epithelium
CEM	-	Cut end of muscles
DC	-	Differentiating cartilage cells
E	-	Ependyma
MC	-	Mesenchymal cells
NC	-	Nerve cord
SBE	-	Stratified blastemic epithelium



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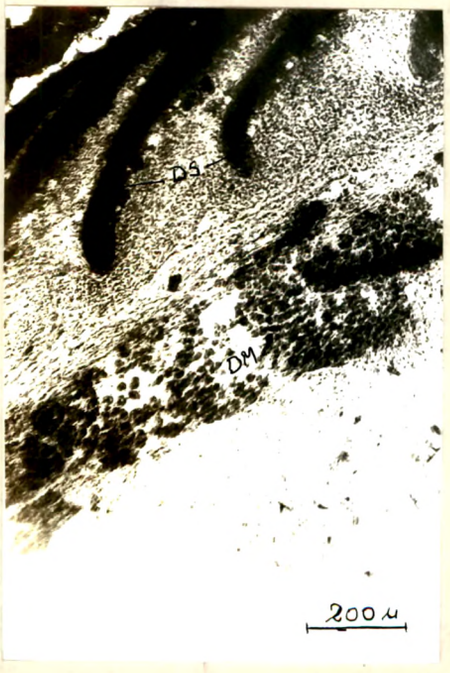

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Fig. 7. Photomicrograph of L.S. of differentiating tail displaying LDH activity in the various differentiating components.

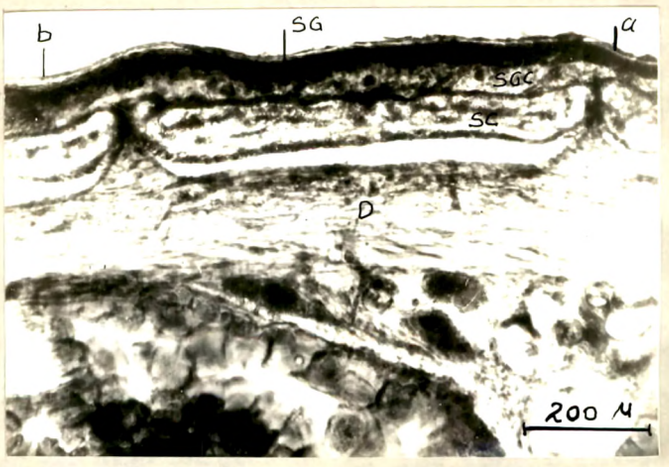
Fig. 8. Photomicrograph of differentiating scales and muscles showing high LDH activity.

Fig. 9. Photomicrograph showing enzyme activity in the cartilaginous neural canal and ependyma.

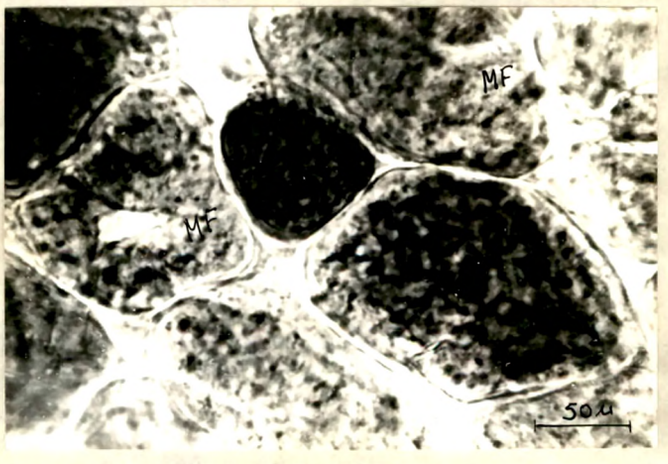
ABBREVIATIONS

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

1A



2A



3A

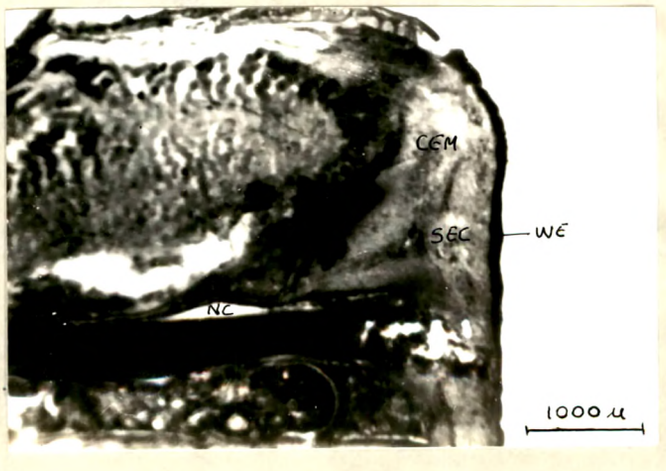


Fig. 1A. Photomicrograph of T.S. of normal tail skin showing MDH activity. Note the activity of MDH in stratum germinativum and scutogenic cells.

Fig. 2A. Photomicrograph of T.S. of normal tail muscles demonstrating MDH activity.

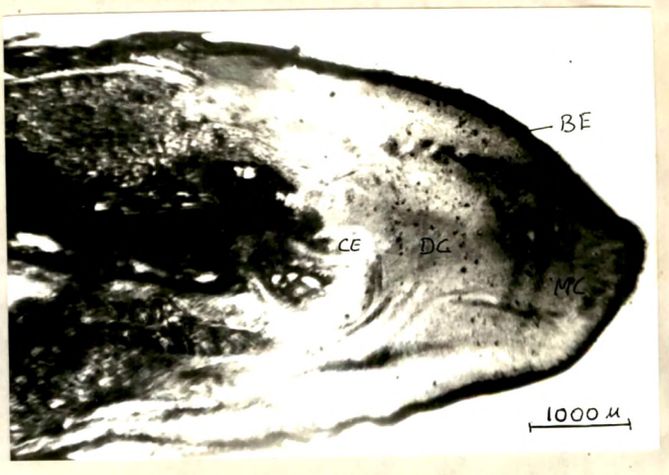
Fig. 3A. Photomicrograph of L.S. of wound healing tail showing MDH activity. Note the enzyme activity in the wound epithelium.

ABBREVATIONS

a	-	alpha cells
b	-	beta cells
CEM	-	Cut end of muscles
D	-	Dermis
MF	-	Muscle fibres
NC	-	Nerve cord
SC	-	Scute
SEC	-	Subepithelial cells
SG	-	Stratum germinativum
SGC	-	Scutogenic cells
VC	-	Vertebral column
WE	-	Wound epithelium

4 A

5 A



6 A

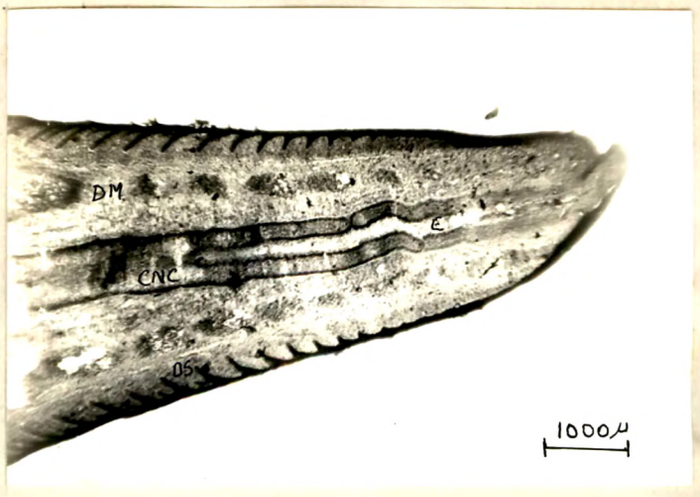
Fig. 4A. Photomicrograph of wound epithelium and subepithelial cells denoting the enzyme activity.

Fig.5A. Photomicrograph of L.S. of blastema showing MDH activity. Note the activity in the blastemic epithelium and mesenchymal cells.

Fig. 6A. Photomicrograph of stratified blastemic epithelium showing enzyme activity. Note the enzyme activity in the subapical mesenchymal cells as well.

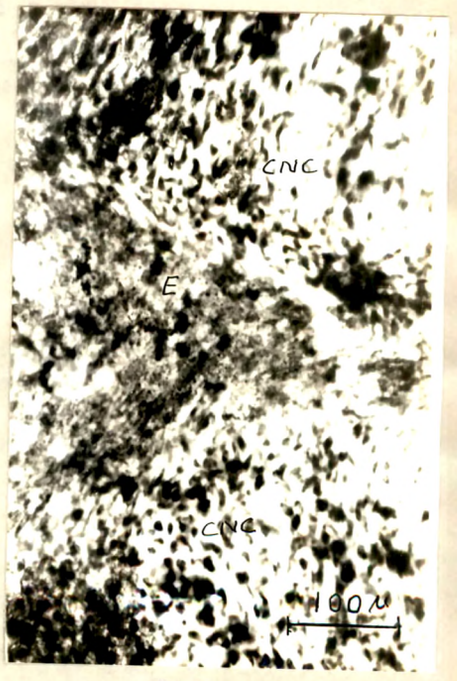
ABBREVIATIONS

BE	-	Blastemic epithelium
CE	-	Cut end of Tail
DC	-	Differentiating carilage
MC	-	Mesenchymal cells
SAMC	-	Subapical mesenchymal cells
SBE	-	Stratified blastemic epithelium
SEC	-	Subepithelial cells
WE	-	Wound epithelium



7 A

8 A



9 A

Fig. 7A. Photomicrograph of L.S. of differentiating tail depicting MDH activity in the various differentiating tissues.

Fig. 8A. Photomicrograph showing high MDH activity in the differentiating scales and muscles.

Fig. 9A. Photomicrograph of the cartilaginous neural canal with ependyma showing enzyme activity.

ABBREVIATIONS

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

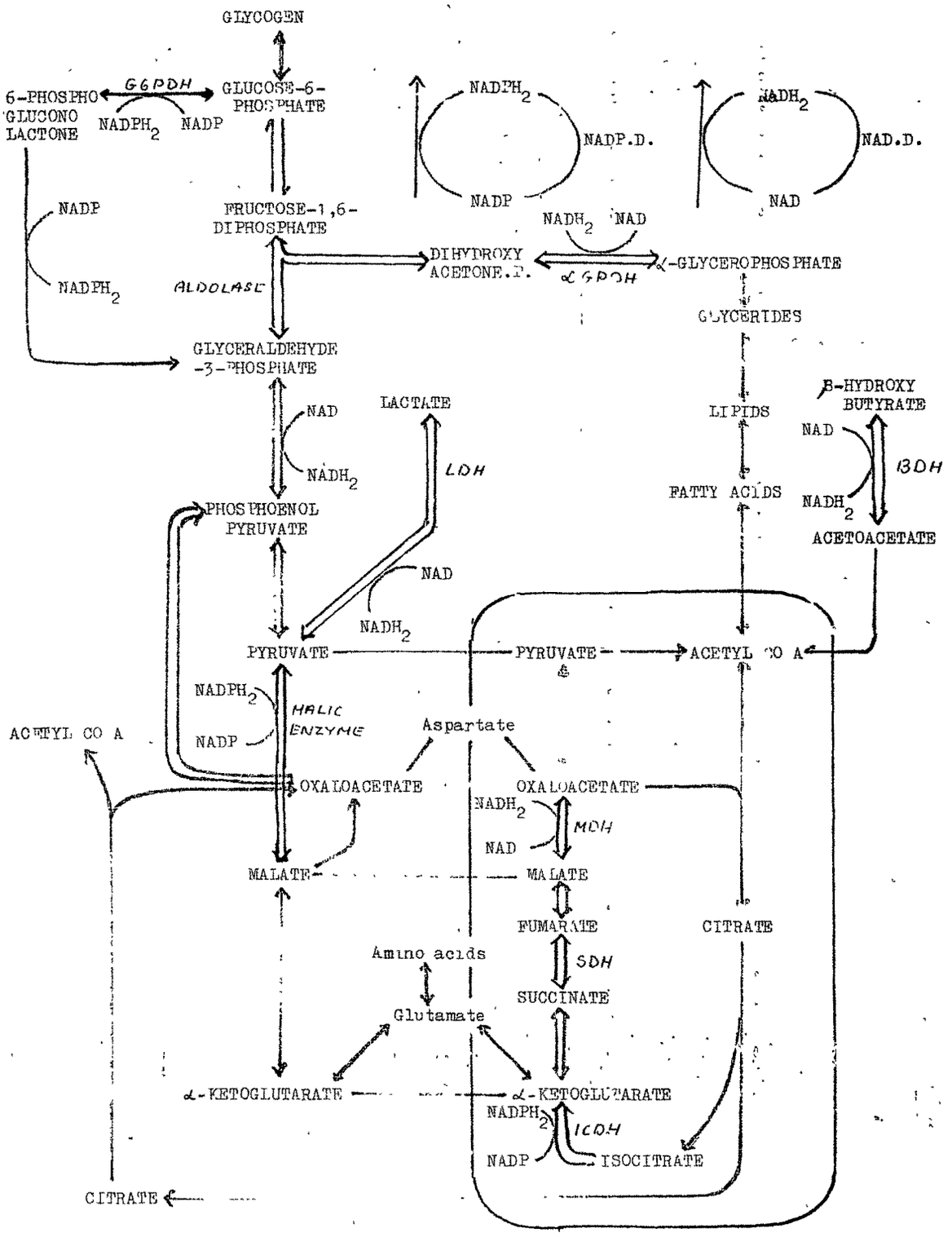
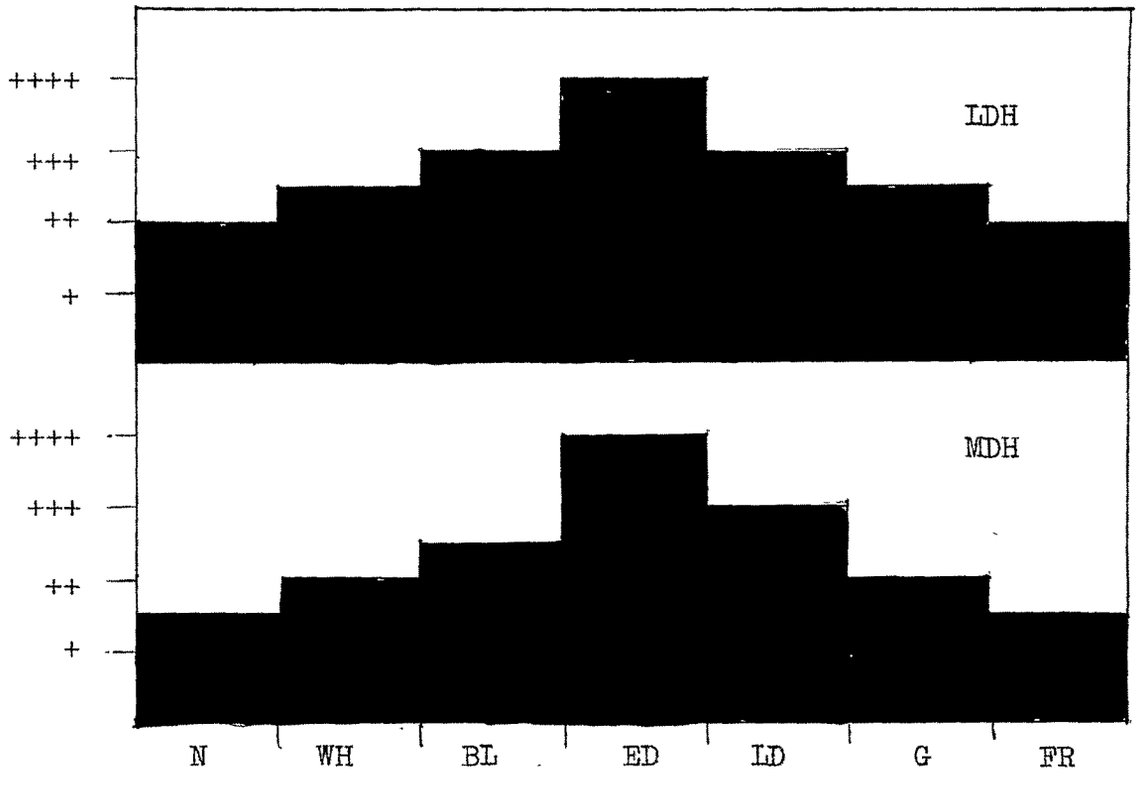

GLYCOGEN
6-PHOSPHO GLUCONO LACTONE
G6PDH
NADPH2
NADP
GLUCOSE-6-PHOSPHATE
NADPH2
NADP.D.
NADP
NADH2
NAD.D.
NAD
NADP
FRUCTOSE-1,6-DIPHOSPHATE
NADH2
NAD
DIHYDROXY ACETONE.P.
α GPDH
α-GLYCEROPHOSPHATE
NADPH2
ALDOLASE
GLYCERIDES
GLYCERALDEHYDE -3-PHOSPHATE
LACTATE
LIPIDS
β-HYDROXY BUTYRATE
NAD
NADH2
LDH
NAD
NADH2
FATTY ACIDS
NADH2
NAD
3DH
ACETOACETATE
PHOSPHOENOL PYRUVATE
NAD
NADH2
PYRUVATE
PYRUVATE
ACETYL CO A
NADPH2
NADP
MALIC ENZYME
ACETYL CO A
Aspartate
OXALOACETATE
OXALOACETATE
NADH2
NAD
MDH
MALATE
MALATE
FUMARATE
CITRATE
Amino acids
SDH
SUCCINATE
Glutamate
α-KETOGLUTARATE
α-KETOGLUTARATE
NADPH2
NADP
ICDH
ISOCITRATE
CITRATE

FIG.3.

Graphic representation of the changes in the LDH & MDH distribution pattern during the various stages of tail regeneration



N: Normal tail; WH: Wound healing phase;

BL: Blastemic phase; ED: Early differentiation phase;

LD: Late differentiation phase; G: Growth phase;

FR: Fully regenerated tail.

progressed from the base towards the apex, the differentiating chondroblasts and myoblasts showed progressive increase in enzyme concentration. ~~All~~ Throughout differentiation, the differentiating scales and epidermis alongwith the differentiating scutogenic cells and muscle fibres were found to maintain a very high enzyme activity.

Growth phase:

During this phase, the differentiated chondrocytes and myofibres attained the level of enzyme activity characteristic of the corresponding cells of the normal tail. The cartilagenous neural canal showed appreciable activity whereas the ependyma was not very active. But during the final stages of growth phase, the ependyma and the gleal cells became enzyme active and within the cartilgenous neural canal, only the central core of chondrocytes showed enzyme activity, whereas the inner and peripheral chondrocytes of the canal displayed a contrastingly poor enzyme activity.

The fully grown regenerated tail revealed an identical intensity and pattern of enzyme activity and localization as seen in the corresponding tissues of the normal tail.

DISCUSSION

The presence of highly active LDH and MDH in any animal tissue generally suggests an active glycolysis and TCA cycle as the most probable routes of metabolism in that tissue. The present observations in the tail of Mabuya carinata are in conformity with the findings of Wolfe & Cohen 1963 and Schmidt & Weidman 1964 who observed a higher activity of these two enzymes has been observed in regenerating amphibian appendages by Wolfe and Cohen (1963) and Schmidt and Weidman (1964). Though MDH was found to be slightly less active as compared to LDH, both these enzymes showed a more or less parallel and identical distribution and localization in the normal as well as the various phases of the regenerating tail. The high activity of MDH, one of the TCA cycle enzymes along with LDH when viewed in the light of reported low activities of the other two important TCA cycle enzymes viz., ICDH and SDH, is highly noteworthy and is suggestive of intriguing metabolic reactions in regenerating vertebrate tissues with pyruvate holding a key position as already hinted by Schmidt (1964).

The role of LDH in the glycolytic metabolism is well established and its activity in different tissues

has been studied by a number of workers who have shown that it is one of the most active dehydrogenases amongst dehydrogenases. Pearse (1960) has suggested the presence of LDH as indicative of anaerobic glycolysis. The high incidence of LDH in the various tissues of the normal tail of the lizard may be indicative of an active anaerobic glycolysis. The observed aldolase activity (Chapter 1) also favours the above contention. Presently The observed high LDH activity in the stratum germinativum and the muscles and the presence of glycogen in these cells of Mabuya carinata (Shah and Radhakrishnan, unpublished), Hemidactylus flaviviridis (Shah and Chakko, 1967) are suggestive of that anaerobic glycolysis as the chief metabolic reactions pathway at work in the normal tail. The above deduction is supported by the observations of Schmidt (1963) on newt epidermis and Mustakallio (1962) on human epidermis.

After autotomy, from wound healing phase through preblastemic and blastemic phases of the regenerate there was a gradual increase in the enzyme activity in the various tissues ~~and~~ which reached a peak value in the differentiation phase. Similar observations has been made with regard to LDH in the regenerating

tail of Triturus viridescens (Geczik and Wolsky, 1959) and in the regenerating limbs of the adult newts (Wolfe and Cohen, 1963; Schmidt and Weidman, 1964). This is correlates with the findings that there is a depletion of glycogen from the cut end of the muscles and other cells through preblastemic and blastemic phases of the regenerating limb of Triturus viridescens (Schmidt, 1960) and the absence of glycogen in the cells during these phases in the regenerating tail of Hemidactylus flaviviridis (Shah and Chakko, 1967) points to a definite anaerobic metabolism. This is further supported by the observation of an increased lactic acid content during preblastemic stages of limb regeneration in the axolotl (Okuneff, 1933) and the increased lactic acid levels during anaerobic glycolysis (Dickens, 1951). The concomitant parallelism noticed in the activity of MDH during the various stages of regeneration by the present workers and the equally active MDH and LDH during differentiation, lends credulence to a possible participation of the reversible reaction between malate and oxaloacetate along with the lactate-pyruvate axis. In this connection, Schmidt and Weidman (1964) have suggested that the functional significance of MDH catalysis in regeneration may lie in a pyruvate centered

metabolism possibly through an abbreviated form of the TCA cycle and they have further postulated a negligible role for the TCA cycle in the regeneration blastema. Further support can be derived for this suggestion from the reports of low ICDH activity by Wolfe and Cohen (1963) and SDH by Geczik and Wolsky (1959); Johnson and Singer (1963); Wolfe and Cohen (1963); Schmidt and Weidman (1964) and Shah and Chakko (1969) in the regeneration blastema. Further significant observations were regarding the high consumption of oxygen (Wolsky in Brachet, 1950), a low respiratory quotient for blastema cells (Ryvkina, 1945) and a high level of anaerobically produced lactate (Okuneff, 1933) and (Schmidt, 1960).

The stratified epithelium, the myoblasts and chondroblasts of the regenerate of the early differentiation phase were noted to exhibit a higher response for both LDH and MDH. Along with the progression of differentiation from the base towards the apex the various differentiating cells registered a constant increase of LDH and MDH activities. The activities noticeable at the late differentiation phase were the highest in comparison to those of other phases and represented a stage of peak metabolic activity. This gradual increase of activities can well be accounted with the development and differentiation of the various tissues

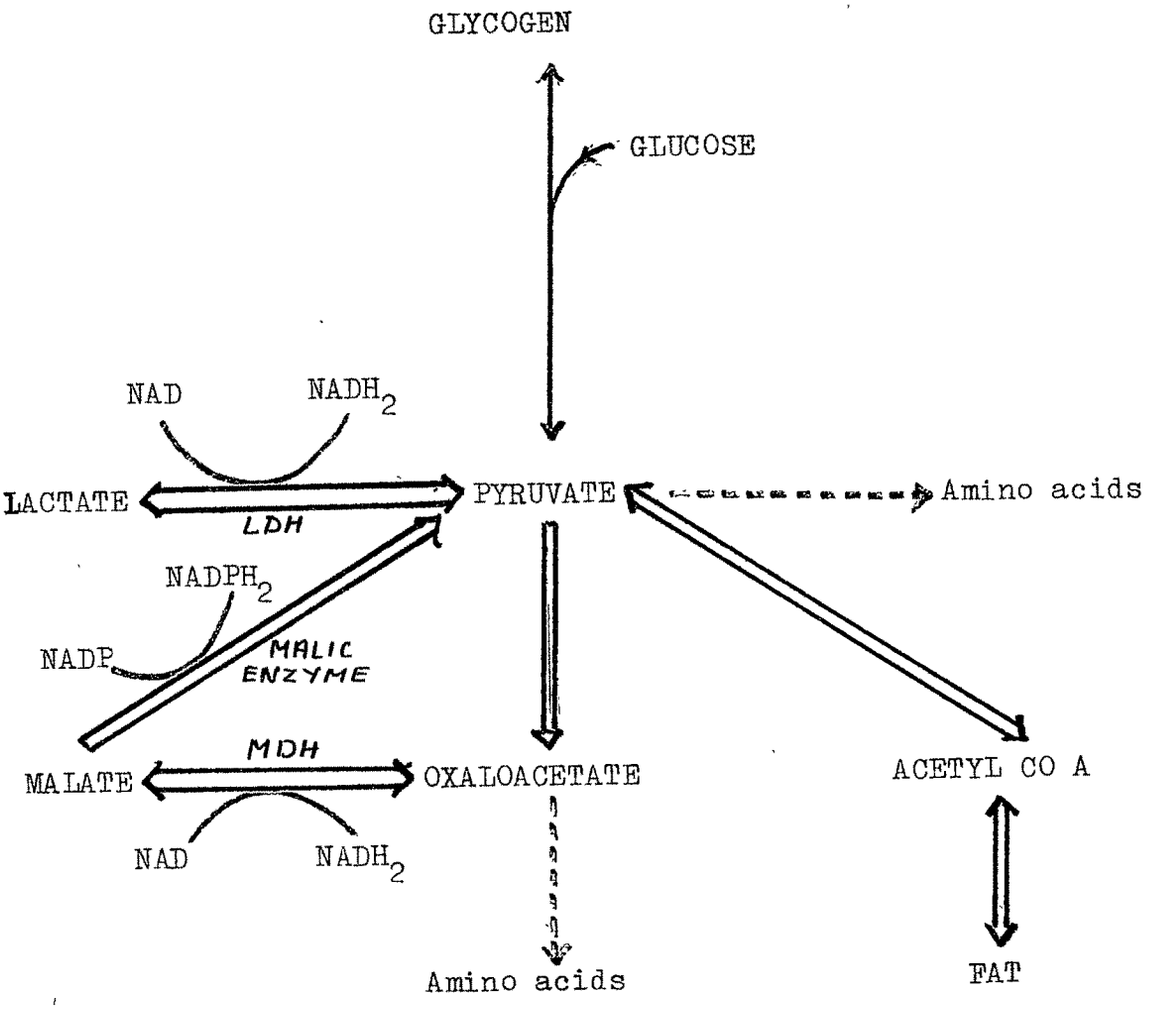
of the tail. The stratified epithelium divides and differentiates into the dermis and epidermis. The potential cells for muscle undergo myogenesis enroute of myoblasts, myocytes and finally myofibres. Chondrogenesis is a similar process taking place simultaneously leading to the formation of cartilage cells. Progressive increase of the enzymes was the characteristic feature in all the above groups of cells. Similar is the observation of Magon (1970) in Hemidactylus flaviviridis. It may be noted here that Shah and Radhakrishnan (unpublished) and Shah and Chakko (1967; 1969) in Mabuya carinata and Hemidactylus flaviviridis respectively have observed a similar pattern of variations with regard to glycogen content and the levels of phosphorylase and SDH activities during differentiation. Similar report on progressive increase of LDH and MDH activities during differentiation come from the studies in the regenerating forelimb of Diemictylus viridescens (Schmidt and Weidman, 1964). These observations exemplify the metabolic adaptations at the subcellular level during differentiation phase.

Apart from the increase of LDH (present chapter) and aldolase (Chapter 1) the increase of MDH

(present chapter) and the acquisition of SDH and ICDH (Chapter 5); (Shah and Chakko, 1969) and cytochrome oxidase (Chapter 6); (Magon, 1970) during this phase highlights the importance of the TCA cycle as a supplementary achievement in addition to anaerobic glycolysis. The concomitant increase of glycolytic enzymes noted herein and of glycogen (Shah and Radhakrishnan, unpublished) and (Shah and Chakko, 1967) is a bit intriguing and may be accounted for by the suggestion of an increased glycogen synthesis rather than a glycogen degradation. It is quite probable that the rate of synthesis and breakdown of glycogen keep pace with each other during early differentiation but the former overruns the latter during late differentiation. But to keep the two processes running at a positive level at the same time calls for additional and or increased carbohydrate mobilization either from blood or some other alternate source mediated through metabolic interconversions (Chapter 4).

Since the chondrocytes during chondrogenesis were found to have detectable levels of glycolytic enzymes i.e., aldolase and lactate dehydrogenase in the present study, and also glycogen (Shah and Radhakrishnan, unpublished) it is surmisable that these

Diagramatic representation of the short pyruvate centered cycle discussed in the text



cells are dependant on glycolysis for their metabolic fulfilments. The enhanced glycolytic activity of the late differentiation phase seemed to step down gradually during growth phase and attain the normal level in the fully regenerated tail. This is reflected in the gradual diminition of LDH and MDH during growth phase until the distribution pattern of these two enzymes in the regenerated tail resembled that of the normal one.

In the wake of these observations, it could be suggested that in the regenerating tail of Mabuya carinata also, a strong pyruvate centered metabolism is in existance, closely linked with lactate, malate and oxaloacetate. It is quite possible that this short cycle is similar to the pyruvate carboxylase shuttle present in the vertebrate liver and muscle .

A close observation of this pyruvate cycle reveals, that a part of pyruvate is converted into lactate for the production of nicotinamide adenine dinucleotide (NAD) required for the glycolysis at the soluble dehydrogenase level, and the other part is converted through oxaloacetate and malate back to pyruvate, yielding sufficient supply of reduced co.enzyme II ($NADPH_2$) which may be utilized for

lipogenesis. A high concentration of MDH in the epithelium and subapical cells during wound healing and preblastema stages and stratified epithelium and blastema cells during blastemic phase, is in correspondence to the high level of lipids reported by Schmidt (1964) and Chakko (1967). The occurence of lipids and MDH in high concentration may suggest the possibility of lipogenesis. It may further be noted that Levy (1961) has observed substantial MDH activity in the lactating mammary glands undergoing active fatty acid synthesis. The role of malic enzyme in lipogenesis has also been stressed by Wise and Ball (1964).

Moreover, since it is shown that oxaloacetate can be converted into phosphoemol pyruvate without first getting converted into pyruvate, the possibility of glyconeogenesis also exists. It is known that oxaloacetate and pyruvate are precursors for the synthesis of aminoacids which could ultimately be used for building up of protiens. Since, the regeneration process also calls for a high protein synthesis, the production of oxaloacetate and pyruvate are of prime importance from which aminoacids are produced by

transamination. If this is so, the fat broken down during the differentiation phase may be of multipurpose value being not only useful for supplying additional energy to the differentiating cells by its oxidation through the Krebs cycle (Chapter 5) but also of importance in glyconeogenesis as well as aminoacid anabolism.