CHAPTER 5

INTRACELLULAR RESPONSE OF SUCCINATE AND ISOCITRATE DEHYDROGENASES (SDH & ICDH) IN THE NORMAL AND REGENERATING TAIL OF THE SCINCID LIZARD, MABUYA CARINATA

Since succinate and isocitrate dehydrogenases play important roles in the oxidative metabolism, the histochemical distribution of these two enzymes usually provides an index to the existence of an active aerobically operated metabolism in the tissues concerned. The localization and distribution of these two enzymes have been undertaken by many workers in many of the vertebrate tissues. Succinate dehydrogenase (SDH) has been more extensively studied than isocitrate dehydrogenase (ICDH) mainly for the reason that it is an enzyme chiefly concerned with the TCA cycle oxidation whereas ICDH is known to play an active role in many other synthetic activities outside the TCA cycle. Cooper (1955) in the prenatal and postnatal rat heart, George, Susheela and Scaria (1958a) in the breast muscles of bat, Goddard and

Seligman (1952) in the thyroid of albino rat, Goddard and Seligman (1953) in rat hepatoma, Padykula (1952) in tissue sections of rat, Rutenburg, Wolman and Seligman (1953) a comparative study in six mammals, and Sternberg, Farber and Dunlap (1956) in kidney, are a few to name, who have studied SDH. In comparison, ICDH has been investigated only by a handful of workers. Geczik and Wolsky (1959) determined SDH using the Thunberg technique on homogenates of the regenerating tail of the newt, Triturus viridescens. Succinate dehydrogenase has been studied in nonregenerating (Schmidt, 1963b) and regenerating (Geczik and Wolsky, 1959; Johnson and Singer, 1964; Niwelinski, 1960; Schmidt and Weidman, 1964; Wolfe and Cohen, 1963) tissues of adult urodeles.

A strong histochemical response has been obtained for SDH in limb epidermis and striated muscles (Schmidt, 1963b). Niwelinski (1960) and Wolfe and Cohen (1963) also reported a marked enzyme response from the limb epidermis and striated muscle. In the regenerating limb of adult Triturus vulgaris, Niwelinski (1960) obtained a weak SDH response from the post amputational wound epithelium, which was followed by a progressive

increase in activity until its intensity equalled the marked response of the limb stump epidermis at ten days. In contrast, both Wolfe and Cohen (1963) and Schmidt and Weidman (1964) reported an intense response in the wound epithelium from 24 hours post amputation and continuing throughout the period of limb regeneration in adult Diemictylus viridescens. Further disparity in results could be made out from the reports on the study of the same enzyme with respect to the regeneration blastema. Geczik and Wolsky (1959) found SDH to be the least active of the dehydrogenases studied by him. There was a preblastemic drop below normal levels in enzyme activity following a brief rise at 24 hours post amputation. However, with the formation of the blastema at the 10th day of regeneration, SDH increased to a peak activity much above the normal level. This marked increase in enzyme activity receives support from Niwelinski's (1960) histochemical results of an intense blastema-cell SDH reactivity. In sharp contrast to these latter two reports are the recent reports by Wolfe and Cohen (1963); Johnson and Singer (1964) and Schmidt and Weidman (1964) of a distinct diminution of SDH activity in the cells forming the regeneration blastema in the amputated limb

of the adult newt. A reappearance of SDH response during the histogenesis of differentiation is reported by Wolfe and Cohen (1963) and Schmidt and Weidman (1964). The only report of the study of ICDH comes from Wolfe and Cohen (1963) in amphibian limb regeneration. They reported ICDH to be a complement of the limb stump epidermis and of the apical wound epithelium. A sharp loss in ICDH activity was noted to be a conspicuous feature of the cells forming the regeneration blastema. The enzyme reappeared by about the fourth to fifth week post amputation in association with the differentiation of muscle and skeleton. The detection of a negligible SDH reactivity in the blastemal cells by Johnson and Singer (1964), Wolfe and Cohen (1963) and Schmidt and Weidman (1964) alongwith the low activity of ICDH (Wolfe and Cohen, 1963) led Schmidt and Weidman to suggest a break in the continuity of the TCA cycle within the cells of the blastema and concluded that TCA cycle per se plays a negligible role in the metabolism of the regeneration blastema.

It was in this background of confusing and contradicting picture available at the moment regarding

Because of the confusion existing on this subject

the role of these two enzymes during regeneration in the amphibian limb and as a continuation of the present study that it was thought pertinent to extend the studies further by investigating histochemically these two enzymes in the regenerating tail of the lizard, Mabuya carinata, so as not only to clear the present picture furthermore but also to draw attention to the possible metabolic reactions involved in the regenerating reptilian tissues and to understand the similarities and or differences if any, in the activity and role of these two enzymes during regeneration in reptiles as compared with that in amphibians.

long sentence!

MATERIALS AND METHODS

The selected adult Mabuyas were maintained in the laboratory on a diet of young cockroaches. The autotomized normal and regenerating tails were cut, blotted to remove blood and tissue fluids and were immediately fixed on a microtome chuck in a cryostat microtome maintained at -20°C. Longitudinal and transverse sections of 12-18 μ thickness were cut and incubated at room temperature for about an hour in the

respective media adjusted to pH 6.5-7 and prepared as follows.

Incubation medium for SDH (After Nachlas, 1957)

Substrate (sodium succinate)	0.2 M	0.5 ml
Tetrazolium salt (Nitro-BT)	1 mg/ml	1.0 ml
Buffer (Phosphate buffer, pH 7.7)	0.2 M	0.5 ml

Incubation medium for ICDH (After Hess, Scarpelli and Pearse, 1958)

Substrate (Isocitric acid, sodium salt)	1.0 M	0.1 ml
Tetrazolium salt (Nitro-BT)	1 mg/ml	0.25 ml
Co.enzyme (Triphospho pyridine nucleotide)	0.1 M	0.1 ml
Respiratory inhibitor (Sodium cyanide)	0.1 M	0.1 ml
Activator (Magnesium chloride)	0.05 M	0.1 ml
Buffer (Phosphate buffer, pH 6.8-7)	0.06 M	0.25 ml
Distilled water		0.1 ml
Polyvinylpyrrolidone		75 mg

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

OBSERVATIONS

NORMAL TAIL (Figs. 1 & 1A)

Skin:

The outer beta and alpha layers of cells of the epidermis showed little or no localization of SDH or ICDH. The stratum germinativum showed noticeable activity whereas the scutes and the dermis showed a negative activity. Even in the stratum germinativum, the activities of these two enzymes when compared to those of other dehydrogenases studied were very much of a lower level.

Muscles:

Muscles showed appreciable activity though not very commentable. The activity was found to be mitochondrial and was seen prominantly only in the smaller peripheral fibres whereas the inner fibres of the fasciculi showed a weak response.

Submuscular and subcutaneous adipose tissue:

No trace of activity of these two enzymes are noticeable in the adipose tissue.

Vertebral column and nerve cord:

The bone matrix and osteocytes of the vertebral column were enzyme negative whereas slight activity was noticeable only in the cartilage cells of the articulating surfaces of centrum. In the nerve cord, the enzymes were poorly localized with only the grey matter showing a slight response.

REGENERATING TAIL

Wound healing phase: (Figs.2, 4 and 2A, 3A)

The wound epithelium showed appreciable though less pronounced activity of SDH and ICDH. Nevertheless, the activity of the enzymes in the cells of the wound epithelium was found to be higher than that observed in any of the tissues of the normal tail.

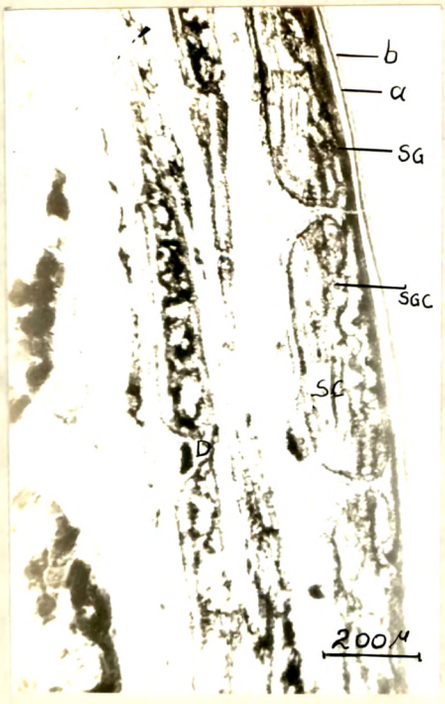
Preblastemic and blastemic phases: (Figs.3-5 & 4A, 5A)

The activity of these two enzymes were seen to show a tendency to increase towards higher levels in comparison to the original tail tissues during the preblastemic and blastemic phases of regeneration. The blastemic epithelium was found to be slightly more enzyme reactive than the wound epithelium. The

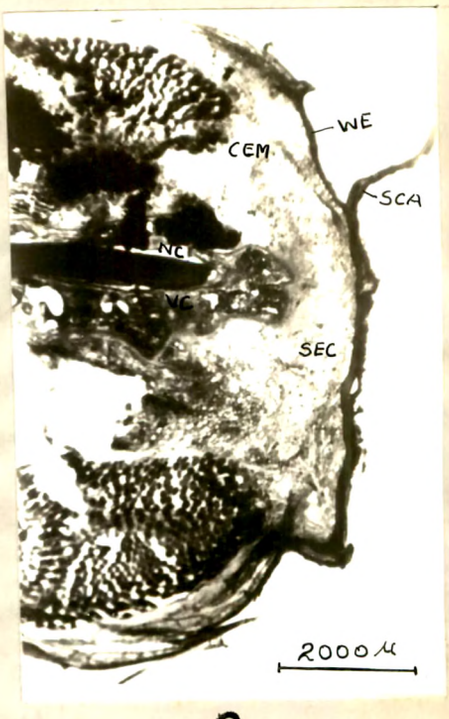
appearance of these two enzymes could be visualized in the form of a bluish tinge taken up by the blastemal cells forming the core of blastema. The blastemal cells though showing more activity than any of the normal tail tissues was much less as compared to LDH, MDH, G6PDH, αC-GPDH and malic enzymes. Eventhough both the enzymes were identifiable in the blastema cells, the ICDH activity was noticeably higher than that observable for SDH.

Differentiation phase: (Figs.6-8 & 6A-8A)

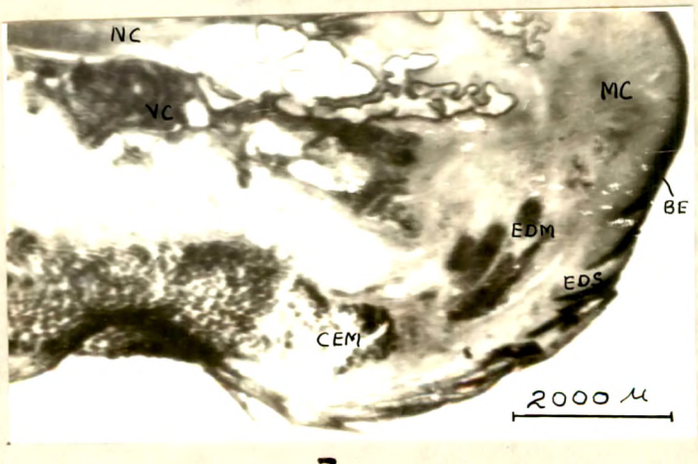
The noticeable SDH and ICDH activities in the blastema, seemed to strengthen further with the onset of histogenesis of differentiation during the late blastemic phase and this increased activity of these two enzymes remained constant throughout differentiation. Though the intensity of activity reached during differentiation phase was not very high in comparison to the other dehydrogenases studied (LDH, MDH, G6PDH, αC-GPDH and Malic enzyme), it was very high when equated with the earlier observed activity of these two enzymes in the normal tail tissues and during the early phases of regeneration. Eventhough, there was a


b
a
SG
SGC
SC
D
200 μ

1


WE
CEM
SCA
NC
VC
SEC
2000 μ

2


NC
VC
MC
BE
EDM
EDS
CEM
2000 μ

3

EXPLANATIONS FOR FIGURES

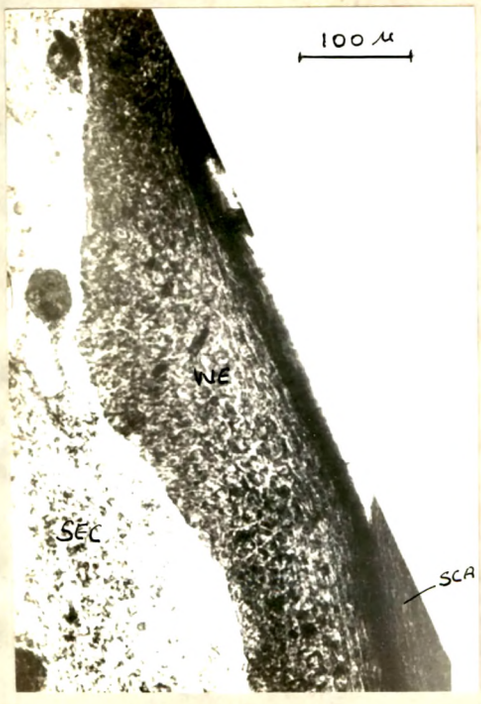
Fig. 1. Photomicrograph of T.S. of normal tail skin showing SDH activity.

Fig. 2. Photomicrograph of L.S. of wound healing tail showing SDH activity.

Fig. 3. Photomicrograph of L.S. of blastema showing SDH activity.

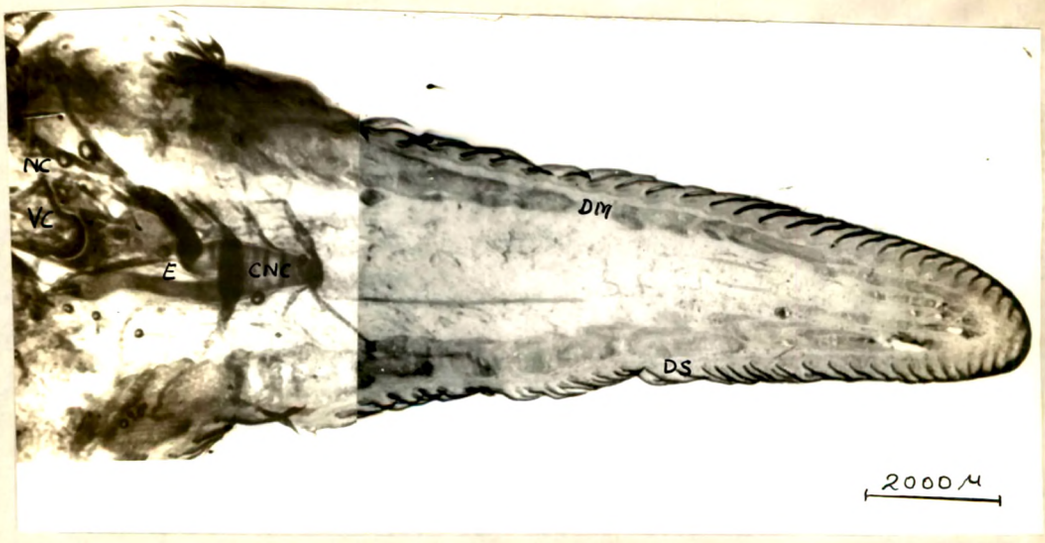
ABBREVIATIONS

a	-	alpha cells
b	-	beta cells
BE	-	Blastemic epithelium
CEM	-	Cut end of muscles
D	-	Dermis
EDM	-	Early differentiating muscles
EDS	-	Early differentiating scales
MC	-	Mesenchymal cells
NC	-	Nerve cord
SC	-	Scute
SCA	-	Scab
SEC	-	Subepithelial cells
SG	-	Stratum germinativum
SGC	-	Scutogenic cells
VC	-	Vertebral column
WE	-	Wound epithelium



4

5



6

Fig. 4. Wound epithelium showing SDH activity.

Fig. 5. Blastemic epithelium showing increasing SDH activity.

Fig. 6. Photomicrograph of L.S. of differentiating tail showing high SDH activity in the various differentiating tissues.

ABBREVIATIONS

BE	-	Blastemic epithelium
CNC	-	Cartilaginous nerual canal
DM	-	Differentiating muscles
DS	—	Differentiating scales
E	-	Ependyma
NC	-	Nerve cord
SAMC	-	Subapical mesenchymal cells
SCA	-	Scab
SEC	-	Subepithelial cells
VC	-	Vertebral column
WE	-	Wound epithelium

7

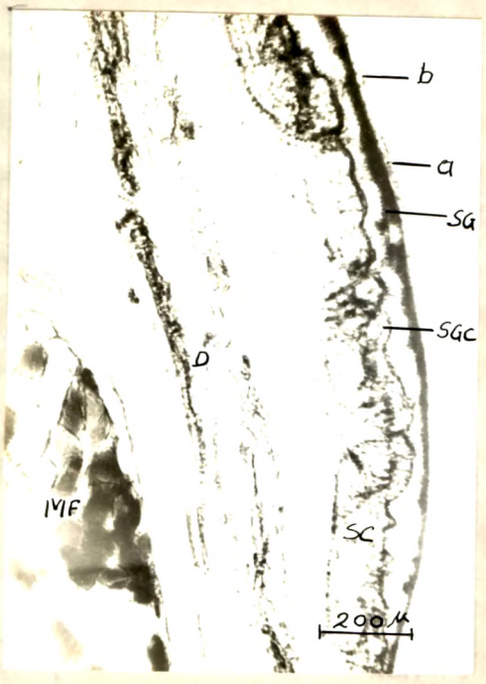
8

Fig. 7. Strong SDH activity in the differentiating scales and muscles.

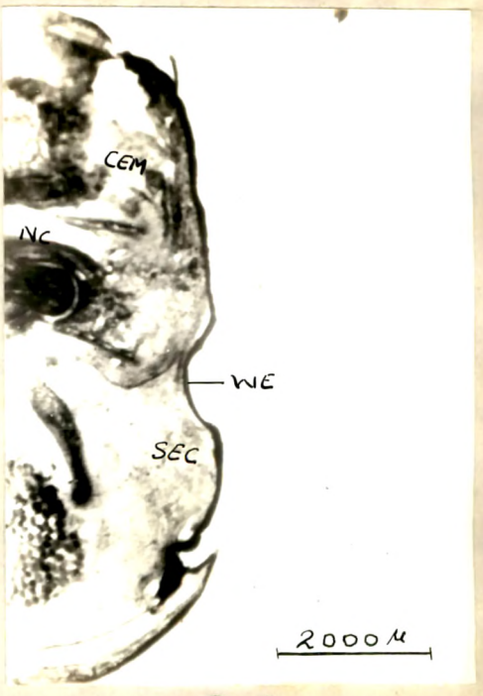
Fig. 8. SDH activity in the cartilaginous neural canal and ependyma.

ABBREVIATIONS

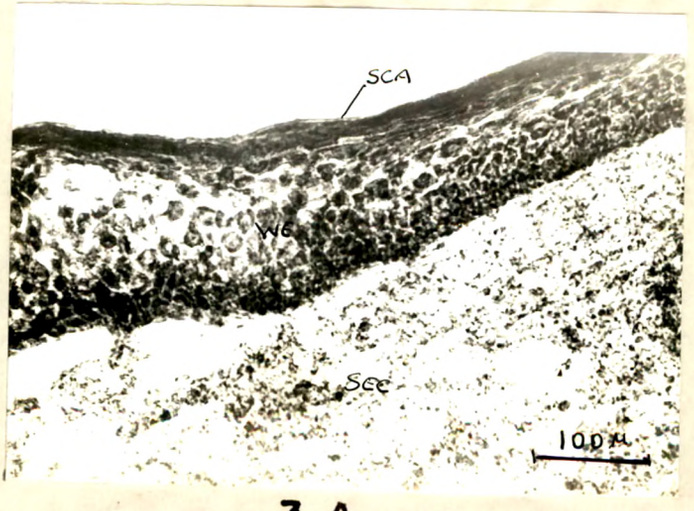
CNC	-	Cartilaginous neural canal
DM	-	Differentiating muscles
DS	-	Differentiating scales
E	-	Ependyma



1 A



2 A



3 A

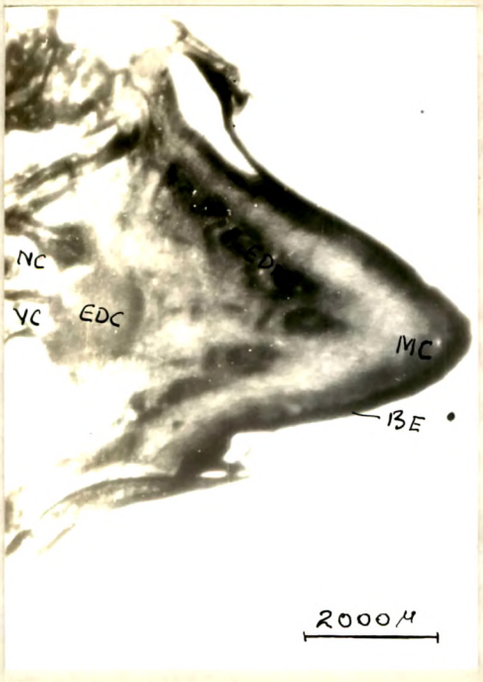
Fig. 1A. Photomicrograph of T.S. of normal tail skin showing ICDH activity.

Fig. 2A. Photomicrograph of L.S. of wound healing tail showing ICDH activity.

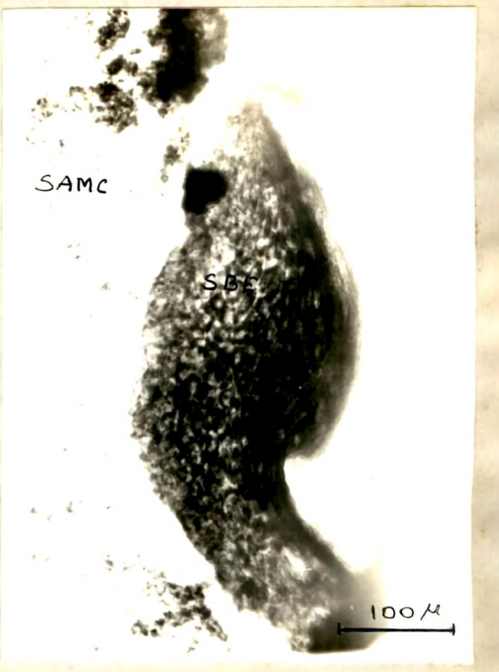
Fig. 3A. Wound epithelial cells depicting ICDH activity.

ABBREVIATIONS

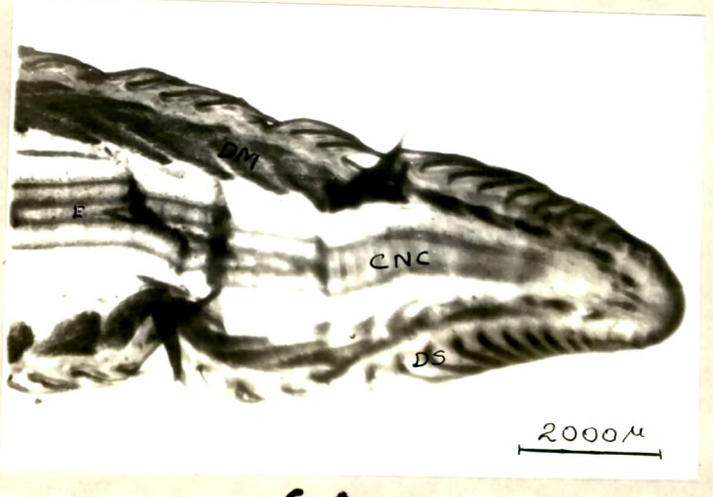
a	-	alpha cells
b	-	beta cells
CEM	-	Cut end of muscles
D	-	Dermis
MF	-	Muscle fibres
NC	-	Nerve cord
SC	-	Scute
SCA	-	Scab
SEC	-	Subepithelial cells
SG	-	Stratum germinativum
SGC	-	Scutogenic cells
VC	-	Vertebral column
WE	-	Wound epithelium



4 A



5 A



6 A

Fig. 4A. Photomicrograph of L.S. of blastema showing increased ICDH activity.

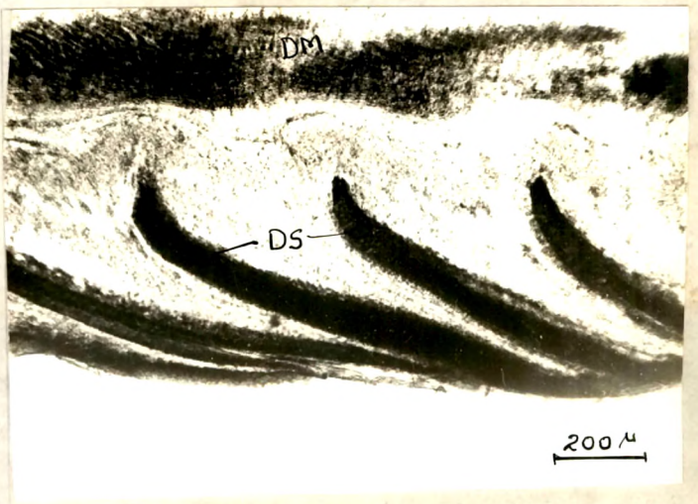
Fig. 5A. Blastemic epithelium eliciting moderate ICDH response.

Fig. 6A. Photomicrograph of L.S. of differentiating tail showing high ICDH activity.

ABBREVIATIONS

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

7A



8A

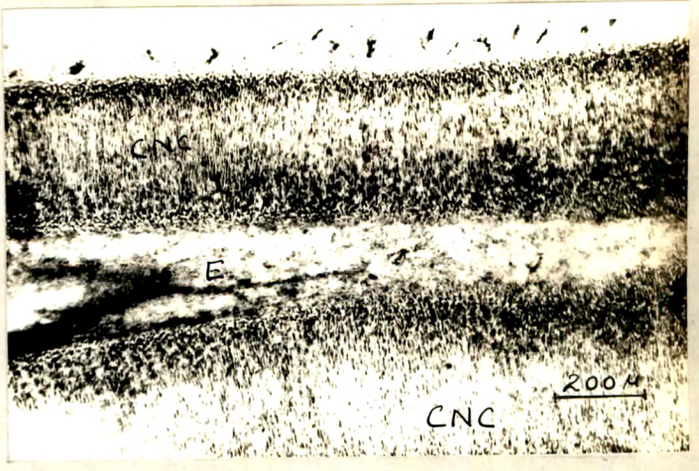


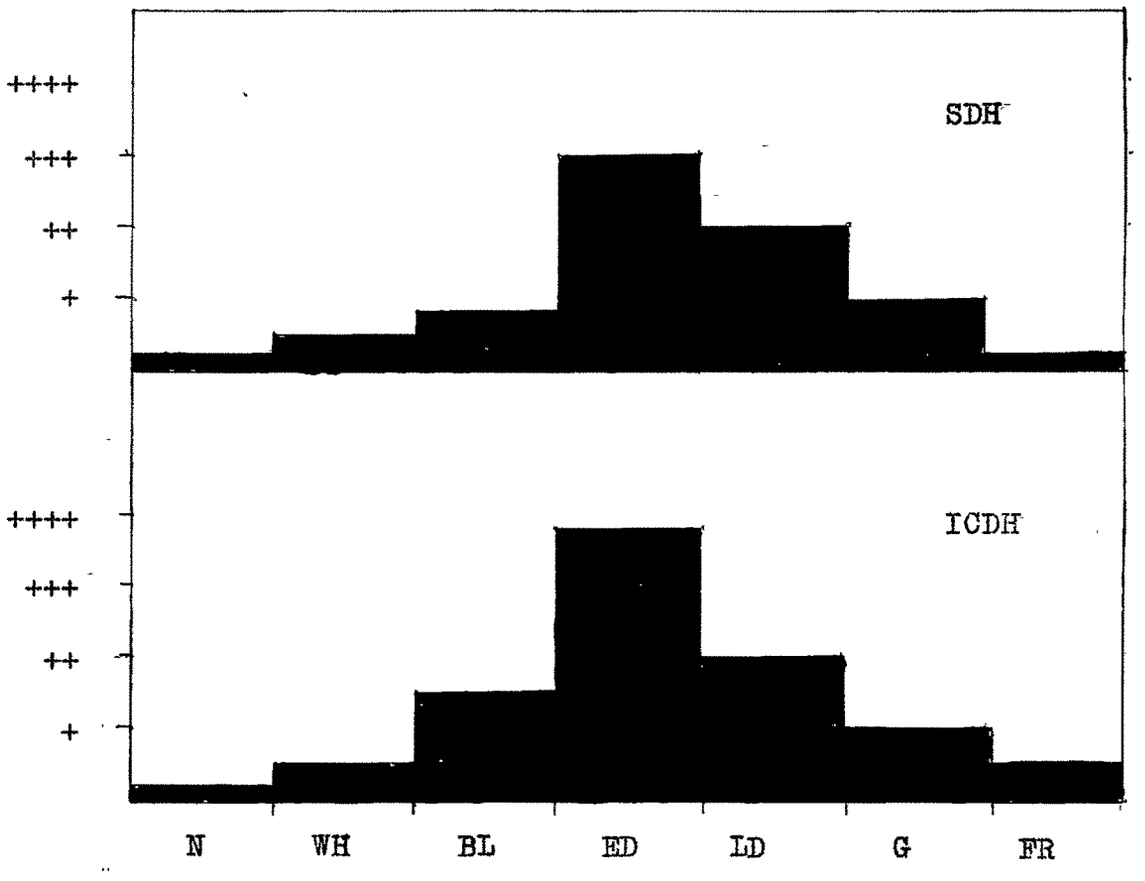
Fig. 7A. Differentiating scales and muscles with enhanced ICDH activity.

Fig. 8A. Cartilaginous neural canal and ependyma showing ICDH activity.

ABBREVIATIONS

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

Graphic representation of the changes in the SDH & ICDH 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.

similarity in the pattern of distribution of these two enzymes, a careful scrutiny revealed the tendency of ICDH activity to be more sharper than SDH. The enzyme response could be noticed in the differentiating scales, muscles and chondrocytes all throughout differentiation.

Growth phase:

With the completion of the differentiative process and the onset of growth phase, there was a gradual fall of SDH and ICDH activity in the various tissues. In the final growth phase, the skin, the muscles, the cartilage cells and the ependyma all showed a much diminished enzyme activity and the full grown regenerate attained the original low level of activity observed in the normal tail tissues.

DISCUSSION

Eventhough, Niwelinski (1960), Wolfe and Cohen (1963) and Schmidt (1963b) obtained a strong histochemical response in the epidermis and striated muscles of the adult urodeles, no such similar strong response in lizard tissues was noticed in the present

work. The weaker activities of SDH and ICDH in the normal tail tissues observed in the present study stengthen the earlier conclusion (Chapter 1) that the normal tail of Mabuya carinata is predominantly anaerobic. The feeble representation of the TCA cycle enzymes noticed herein seems to be in good correlation with the observed poor lipid content in the normal tail (Shah and Radhakrishnan, unpublished). SDH has been demonstrated in the stratum germinativum of the mammalian skin by a number of workers (Padykula, 1952 and Buno and Germino, 1958, in rats; Formisano and Montagna, 1954, in guinea pig; Montagna and Formisano, 1955 and Mustakallio, 1962, in man). Unlike the above observations the normal skin of the tail of Mabuya carinata failed to show a prominant activity. Nevertheless, of all the components of the skin, the cells of stratum germinativum showed relatively more appreciable enzyme concentration. A similar low response was obtained for ICDH as well. Poor localization of lipids and lipolytic enzymes such as lipase and esterase in the normal tails of Mabuya carinata and Hemidactylus flaviviridis (Shah and Radhakrishnan, unpublished and Shah and Chakko, 1967)

and the high incidence of glycolytic enzymes such as aldolase, LDH and ∝-GPDH in both the lizard tails (Chapters 1, 2, 4 and Magon, 1970) when reflected in the light of presently noted poor SDH and ICDH activities emphasize the fact that TCA cycle per se plays a negligible role in the metabolic adaptations of the normal tail of Mabuya carinata, and the weak activities of the same enzymes currently noted in the stratum germinativum and muscle fibres may be looked upon as only presenting a normal complement of TCA cycle enzymes endowed in these cells which if at all might be operating at a very low level, and contributing insignificantly towards the normal tail energetics.

In the regenerating tail the present study indicated a pronounced SDH and ICDH activity in the wound epithelium which continued to register a gradual increase during its stratification and the formation of blastemic epithelium with the ultimate high activity being reached in the differentiation phase. Similar observations have been made with regard to the epithelium of the regenerating limb by Niwelinski (1960) in Triturus vulgaris and by Wolfe and Cohen (1963) and Schmidt and Weidman (1964) in Diemictylus viridescens.

But there was a disparity in results when one considers the reports bearing on the SDH activity of the blastema cells. Geczik and Wolsky (1959) observed the attainment of a peak value of activity of SDH in the blastema. This marked increase in activity received support from Niwelinski's (1960) histochemical result of an intense blastema-cell SDH reactivity. In contradiction to these reports were those of Wolfe and Cohen (1963); Johnson and Singer (1964) and Schmidt and Weidman (1964) of a distinct diminution of SDH activity in the cells forming the regeneration blastema. Wolfe and Cohen (1963) agreed on a similar pattern of ICDH activity too in the blastema. At the same time in the blastema of Hemidactylus flaviviridis Chakko (1969) found no activity of SDH. But in the present study, conducted on the regenerating tail of Mabuya carinata neither an increased nor a diminished activity of SDH and ICDH could be noticed in the mesenchymal cells of blastema as held by the two schools of observations mentioned above. The activity of the enzymes seemed to increase beyond this stage. climb up from here onwards and attain the highest histochemical sensitivity during differentiation. Similar observations are reported

in the regenerating limb of the adult newts by Wolfe and Cohen (1963) and Schmidt and Weidman (1964). Concomitant depletion of glycogen from postamputation phases (Shah and Radhakrishnan, unpublished; Shah and Chakko, 1967; Schmidt, 1960) and the appearance of lipids in the cells of blastema and early differentiating tail (Shah and Radhakrishnan, unpublished; Chakko, 1967; Schmidt and Weidman, 1964) are the convincing evidences in favour of the operation of TCA cycle oxidations at its best? at this stage of regeneration. Later during the late differentiation and growth phases there is a gradual diminution of SDH and ICDH activities along with a lipid exhaustion and glycogen deposition. If the activities of SDH and ICDH are be any index, then, the TCA cycle seems to operate at its maximum at this period of regeneration as both prior to as well as after differentiation the cycle appears? tends to play a negligible role.

The above observations lead to the conclusion that with the onset of histogenesis of differentiation the active anaerobic glycolytic cycle fails to keep pace with the high metabolic

necessities of the cells which are in a tremendous rate of proliferation and hence it is supplemented by the active participation of TCA cycle, ~~which~~ This successfully negotiates the available lipids thus helping to circumvent the extra energy necessities of the actively proliferating cells which are in a process of morphological and physiological differentiation and maturation. With the completion of these processes the energy ~~necessity~~ needs also falls and this is clearly indicated by the general liquidation of lipid content and the concomitant ~~decreasing~~ decrease in SDH and ICDH activities. The utilization of lipids by some other modes also cannot be ignored especially during preblastemic and blastemic phases when TCA cycle seems to be at its ~~infancy~~ lowest levels.

Further, the stronger activity of ICDH in comparison of SDH draws ~~the~~ attention to the possible implication as it is not difficult to surmise when one considers the importance of ICDH and the product of its catalysis in the field of metabolism. It is rather evident that ICDH and malic enzyme both act as satellite enzymes to the HMP shunt in the production

what confusion!

of $NADPH_2$ utilizing NADP. It is also to be noted that α-ketoglutarate the product of ICDH catalysis can easily be converted into certain amino acids by transamination which may thus help in protein anabolism. In addition α-ketoglutarate together with malate are important sources of extramitochondrial oxaloacetate which can lead to glyconeogenesis. Thus it could be safely assumed that ICDH plays an important role in (1) $NADPH_2$ production, (2) glyconeogenesis and (3) protein synthesis, apart from its normal role in TCA cycle oxidations.

With the completion of growth and the attainment of the fully regenerate condition, the SDH and ICDH activities also reached too acquired the low level which was evidenced in the corresponding normal tail tissues.