CHAPTER 4

HISTOCHEMICAL LOCALIZATION OF SUCCINIC DEHYDROGENASE (SDH) IN THE NORMAL AND REGENERATING TAIL OF THE HOUSE LIZARD, *HEMIDACTYLUS FLAVIVIRIDIS*

Geczik and Wolsky (1959) studied the activity of dehydrogenases using the Thunberg technique on tissue homogenates during the regeneration of the tail of *Triturus viridescens* and compared it with the adult tissues. They found that SDH was the least active enzyme amongst the dehydrogenases studied. Though the total dehydrogenase activity in regenerate tissues was 20 to 50% lower than that of the adult tissues during the earlier stages, a high peak in the SDH activity (140% above the adult level) was recorded in the later stages.

A strong histochemical response was obtained for SDH by Schmidt (1963) in the limb epidermis and the striated muscle of the adult newt *Diemictylus viridescens*, by Wolfe and Cohen (1963) in the regenerating Urodele limb and Niwelinski (1960) in the fore limb of the newt, *Triturus vulgaris*. Niwelinski (1960) reported that the SDH activity in the post amputational wound epithelium of the regenerating limb of adult *T. vulgaris* progressively increased until its intensity equalled the SDH activity of the limb stump epidermis on the 10th day. However, Schmidt and Weidman (1964) and Wolfe and Cohen (1963) reported a constant enzyme response

in the wound epithelium from 24 hours post amputation and continuing throughout the period of limb regeneration in adult newt, D. viridescens and in a Urodele respectively. In view of these conflicting reports regarding the dehydrogenase activity in the regenerating appendages, it was thought desirable to investigate histochemically the SDH activity in the various tissues of the normal and regenerating tail of the house lizard, Hemidactylus flaviviridis.

MATERIALS AND METHODS

The normal tail and the regenerates with at least one or two segements of the original tail stump were cut, blotted to remove blood and tissue fluids and kept on a microtome chuck in a cryostat maintained at -20°C. Longitudinal as well as transverse sections of 8 µ were taken and the localization of SDH was studied according to the improved method of George and Talesara (1961) using neotetrazolium as the hydrogen acceptor. The control sections were either heated at 70 to 80°C before incubation or were incubated in a substrate blank medium.

OBSERVATIONS

NORMAL TAIL:

In the epidermis, moderate SDH activity was observed in the cells of stratum intermedium and stratum germinativum.

In the dermis the connective tissue fibres, blood vessels, blood cells and nerve fibres did not reveal any enzyme activity. However, the fat cells of the subcutaneous adipose tissue possessed a weak enzyme activity.

The skeletal muscle fibres showed the highest enzyme reaction amongst all the tissues of the tail. The enzyme was found to be localized in the sarcoplasm and in mitochondria (Fig.1). On the basis of the intensity of the enzyme activity and the number of mitochondria present, three types of muscle fibres could be differentiated - (1) fibres which had a large number of mitochondria and high enzyme activity (mitochondrial as well as sarcoplasmic), (2) fibres which had very few mitochondria and poor enzyme activity and (3) fibres having intermediate values for mitochondrial content and intensity of enzyme activity. It may be noted that such a differentiation of muscle fibres (tail and trunk muscles) ~~was~~ is also noticed when alkaline phosphatase activity in the muscle ~~was~~ is studied (Chapter 7). The cytoplasm of the fat cells of submuscular adipose tissue showed a weak enzyme activity.

The osteoclasts, osteoblasts and osteocytes of the caudal vertebrae and the chondrocytes of the intervertebral cartilage did not have any SDH activity. However, the fat cells and the marrow cells present in the marrow of the vertebral centra and spines did show moderate enzyme activity.

The spinal cord showed uniformly moderate enzyme activity in the gray and white matter, but the peripheral nerves did not have any enzyme activity.

REGENERATING TAIL:

Wound healing phase:

The cells of the wound epithelium contained low SDH activity in the cytoplasm and mitochondria (Fig.2). The subapical cells like erythrocytes, microglial cells of the spinal cord, melanocytes and macrophages had no SDH activity. The enzyme activity in the different tissues of the tail stump remained the same as that noticed in the normal tail.

Preblastemic phase:

At this stage, the wound epithelium increased in thickness by further addition of a few cell layers. The intensity of the SDH activity in the epithelial cells was the same as that in wound healing period. The different types of cells accumulated beneath the epithelial layers were devoid of enzyme activity as in the previous period.

Blastemal phase:

The stratified epithelium of the blastema showed similar SDH activity as noted in the preblastemic phase (Fig.3). The mesenchyme cells were devoid of enzyme.

Late blastemal phase:

During this phase the epidermal cell layers of the skin, all over the regenerate were well differentiated as the stratum germinativum, beta and alpha cell layers. The SDH activity was noticed only in the cells of the stratum germinativum (Fig.4). The epidermal basement membrane did not show any activity. At this stage however, the mesenchyme cells at the base of the regenerate showed differentiation into myoblasts, chondroblasts and ependymal tissue. The myoblasts and chondroblasts were negative to the enzyme reaction but the ependymal tissue showed a low, but uniform enzyme activity. There was a low SDH activity in the fat cells at the proximal part of the regenerate which was more perceptible than that observed in the fat cells of the distal part. The blood capillaries and blood cells did not show any SDH activity.

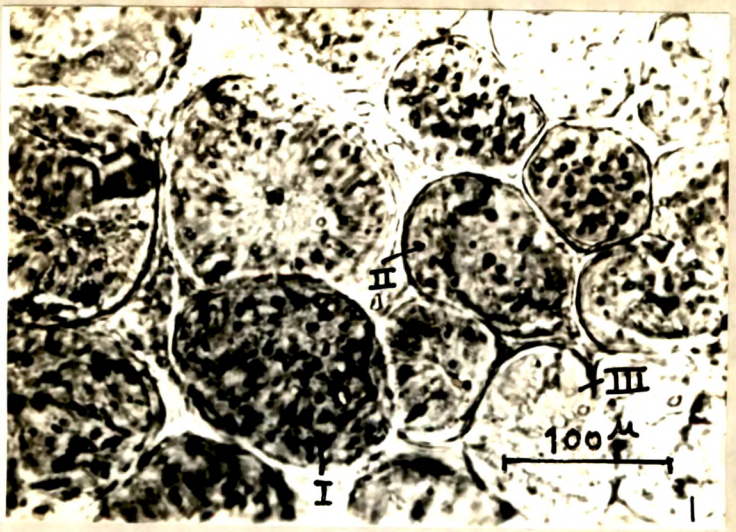
Differentiation phase:

At this stage the formation of epidermal scales had started and the SDH activity reached more or less the same level as it was in the cells of stratum germinativum of the epidermis in the normal tail. The beta and alpha cell layers, the basement membrane and the dermis were negative to the enzyme reaction. During myogenesis, the myoblasts, myocytes and early myofibres did not show the enzyme activity. Similar was the enzyme response in the chondroblasts

and the chondrocytes during chondrogenesis. The blood vessels and the capillaries in the regenerate also did not show SDH activity. However, the ependymal tissue and the fat cells of the subcutaneous and submuscular adipose tissues showed weak SDH activity.

Growth phase:

During the growth phase (till the regenerate reached the length of normal tail) the skin moulted a few times. In the moulting phase though the mitotic activity of the cells of the stratum germinativum was high, the intensity of the enzyme remained the same as it was in the cells of the same layer in the normal tail. There was no enzyme activity in the other cell layers of the epidermis. As in the normal tail, the cells of the dermis and the basement membrane in fully regenerated tail also did not show any SDH activity. In the regenerated muscle, the enzyme activity appeared first in the cytoplasm of the myofibres which were just differentiated. Later the activity appeared in the mitochondria also (Fig.5). The enzyme activity gradually increased as the fibres grew and acquired maturity, reaching more or less the same level as that observed in the myofibres of the normal tail muscle. In the regenerate also, three types of myofibres could be ~~differentiated~~ distinguished based on the SDH activity and their mitochondrial content. The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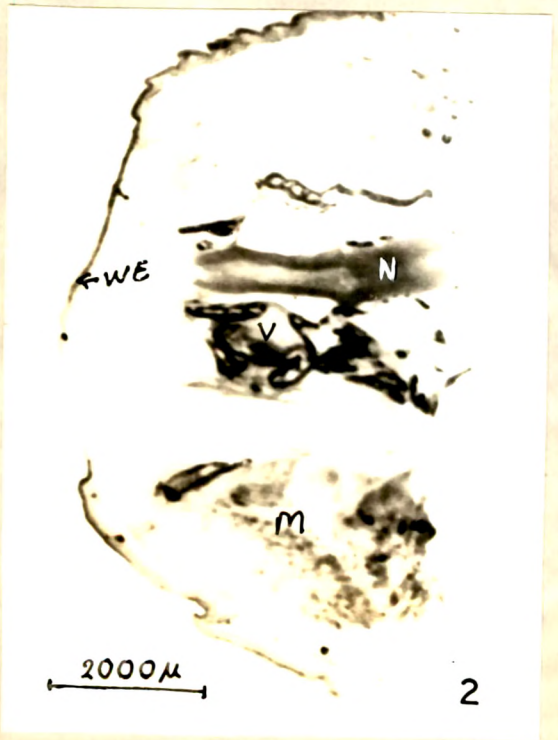
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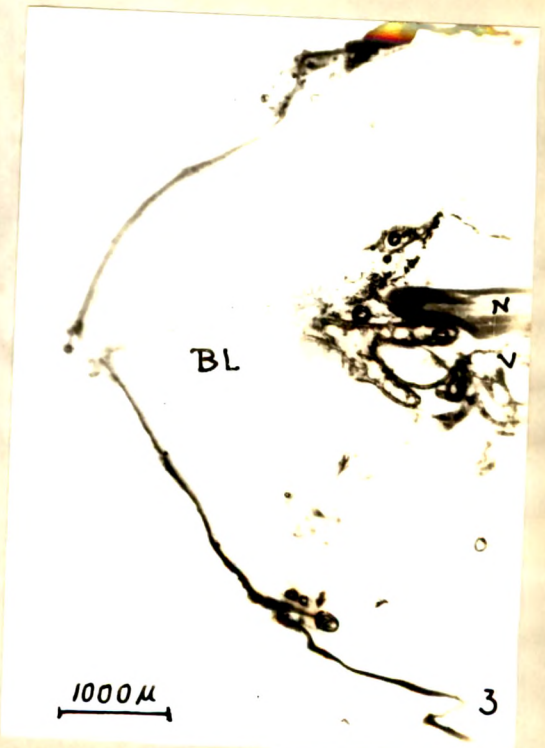
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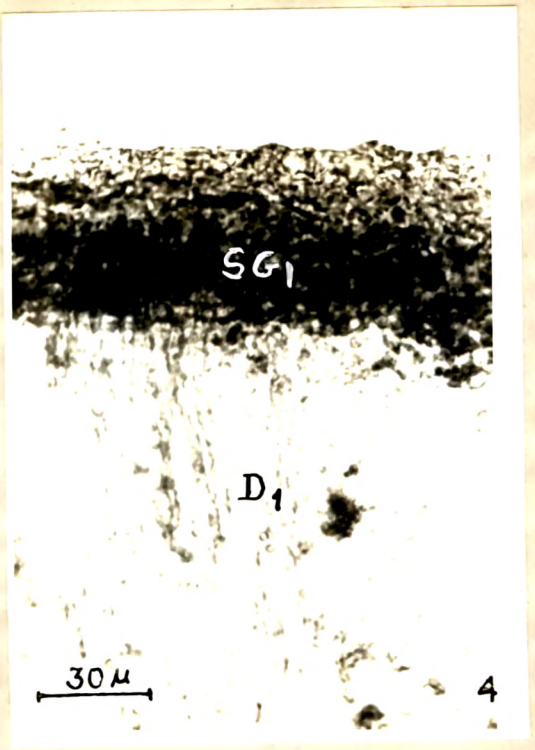
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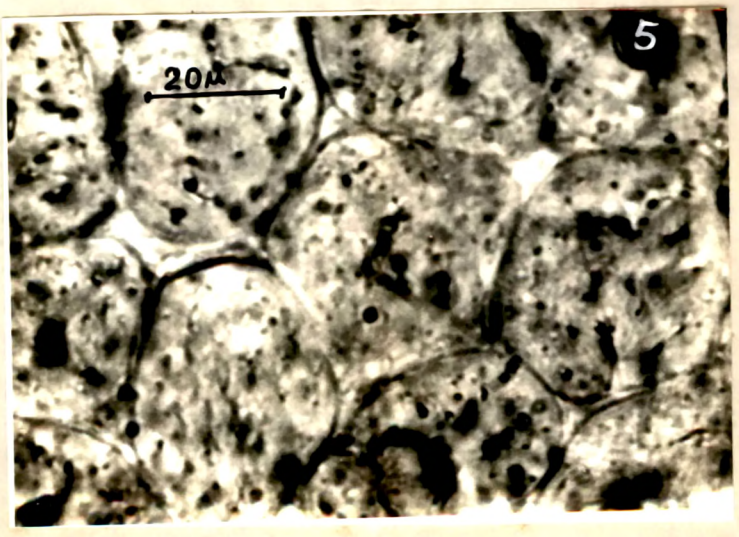
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EXPLANATIONS FOR FIGURES

Fig. 1. T.S. of caudal muscle of the normal tail showing SDH activity in the three types of muscle fibres. I, II, III.

Fig. 2. L.S. of the tail (wound healing phase) showing poor SDH activity in the wound epithelium.

Fig. 3. L.S. of the blastema, showing poor SDH activity in the epithelium. No SDH activity in the subepithelial region is noted.

Fig. 4. L.S. of the skin in the regenerate (differentiation phase) showing SDH activity in stratum germinativum.

Fig. 5. T.S. of caudal muscle (early differentiation phase) where there is no distinction of fibre types. Note low SDH activity in the sarcoplasm and very few mitochondria.

ABBREVIATIONS

BL - Blastema

D_1 - Dermis in the regenerate

M - Muscle

N - Nerve cord (spinal cord)

SG_1 - Stratum germinativum in the regenerate

V - Vertebral column

WE - Wound epithelium

I,II,III - Three types of muscle fibres

ependyma which grew as a thin extension of the spinal cord in the fully regenerated tail showed a uniform distribution of low enzyme activity. Compared to the enzyme activity in the spinal cord of the normal tail it was noticeably low.

DISCUSSION

The presence and distribution of SDH activity in the skin of various mammals have been studied by a number of authors, 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. These studies showed that in the epidermis the SDH activity was most abundant in the stratum germinativum, lesser in spinosum and absent in stratum corneum. The present observations showed that in the epidermis of the house lizard, H. flaviviridis, the SDH activity was restricted to the cells of the stratum intermedium and stratum germinativum. It may be noted that as in the mammals the SDH activity is more localized towards the basal layers of the epidermis. The distribution of the SDH activity is in contrast to that in the amphibian epidermis where in the newt, D. viridescens, it has been reported (Schmidt, 1963) that the basal cells showed somewhat lesser activity than the cells of the successive

outer layers. This variation could probably be related to the fact that the outer layers of the mammalian and reptilian epidermis are more or less inactive, whereas, those of the amphibian skin are not, since most of the amphibians respire through their skin and the outer layers of the epidermis are of active cells.

The present study showed that SDH activity was almost nil in the dermis of the lizard. However, SDH activity was reported in the mammalian fibroblasts (Ogawa and Okamoto, 1961). A poor but consistent SDH activity was also reported in the dermis of the adult newt D. viridescens (Schmidt, 1963).

SDH activity during regeneration has been studied in mammals and amphibia by several workers. However, no studies are reported on the regenerating reptilian tissues.

The postamputational wound epithelium of all the animals studied showed the presence of SDH activity. In the mouse skin it was moderate and more or less similar to that noted in the stump epidermis (Argyris, 1956), in T. vulgaris it was weak in the beginning and progressively increased until in the 10th day old regenerate it reached the level obtained in the limb stump epidermis (Niwelinski, 1960), in the Urodele limb (Wolfe and Cohen, 1963) and D. viridescens (Schmidt and

Weidman, 1964). Intense SDH activity was found at 24 hours post amputation which continued at the same level throughout the entire period of regeneration. In the present study a weak enzyme activity which was less than that in the stump epidermis was first observed in the wound epithelium between 24 hours to 48 hours post amputation time, and that remained unchanged till differentiation of the epidermis commenced.

In amphibians though an increase in the SDH during blastemal period was reported by Geczik and Wolsky (1959) in T. viridescens; a diminution was recorded in the SDH activity by Wolfe and Cohen (1963), Johnson and Singer (1963) and Schmidt and Weidman (1964). In H. flaviviridis, no SDH activity was noticed in the mesenchyme cells though a weak activity was present in the epithelium during the blastemal phase. It may be noted that the mesenchyme cells are actively dividing at this stage and therefore must perform considerable metabolic activity. It may be also mentioned here that in these cells lipid was found to be present but no glycogen and phosphorylase was detected. If the lipids provide the energy for the activity of these cells the absence of SDH cannot be explained. However, it may be possible that the lipids are utilized through other pathways which do not demand TCA oxidation as suggested by Schmidt (1963) and Bueding and Farber (1960).

An increase in the SDH activity was observed in those cells of the stratified epithelium which underwent differentiation into cellular layers of the epidermis. There was a progressive increase in the enzyme activity till all the layers of the epidermis in the regenerate were fully differentiated and scales were formed. Hereafter the enzyme activity was restricted to the cells of the stratum germinativum and reached the same level of activity as was observed in the epidermis of the normal tail. It may be mentioned here that in the present study it was noted that the fat was localized in the cells of the stratum germinativum. Thus the presence of oxidative enzymes suggests that fat is being actively utilized for the metabolic activity of these cells which are periodically forming new generations of beta and alpha cells as the old ones are moulted.

On the basis of the SDH activity and number of mitochondria, three types of muscle fibres could be ~~differentiated~~ distinguished in the tail muscles of H. flaviviridis. Based on similar criteria the muscle fibres in mammals, birds, reptiles and amphibians were also differentiated (George and Talesara, 1961; Stein and Padykula, 1962; Ogata and Mori, 1963). In the regenerating muscle tissue of the house lizard tail, the mononuclear myoblasts showed almost negligible SDH activity. But as they became multinucleated, the enzyme activity first appeared in the

cytoplasm and later in the mitochondria. In the former and particularly in the latter region it gradually increased as morphological and functional maturity of the myofibres progressed. Similarly, Chinoy and George (1965) have shown that SDH activity in the just differentiated myofibres of the pigeon embryo was very poor but upon hatching as the myofibres acquired the morphological and functional maturity, the enzyme also gradually increased in its intensity. In the fully regenerated tail, the muscle fibres presented a similar pattern and intensity of SDH activity as in the normal tail.

The bone tissue of the caudal vertebrae and the intervertebral cartilage in the house lizard was devoid of SDH activity. However, a high enzyme reaction was noticed in the fat and marrow cells which were present in the hollow of the centra and spines. Schmidt and Weidman (1964) have reported that in D. viridescens, this enzyme was just detectable in the chondroblasts but became more evident in osteogenic centres and finally was localized in the periosteum of the regenerating skeletal elements. SDH activity in the chondroblasts of the regenerating tail of house lizard was negligible, however during differentiation and fully grown phases a low but perceptible enzyme activity was seen in the perichondrial and endochondrial regions of the cartilagenous neural canal.

The spinal cord of the normal lizard tail showed low but uniform SDH activity. Malaty and Bourne (1953) reported very poor localization of the enzyme in the mammalian peripheral nerves as compared to the other tissues investigated. Padykula (1952), Buno and Germino (1958), and Thomas and Pearse (1961) reported an absence of SDH in the peripheral nerves of mammals. In the regenerating house lizard tail the ependyma showed slightly lower enzyme activity than what was observed in the spinal cord of the normal tail whereas the peripheral nerves were devoid of SDH. The blood cells and blood vessels of the normal and regenerating tail did not reveal any SDH activity whereas blood cells and the arteries of D. viridescens were reported to show a weak SDH response (Schmidt, 1963).

From the present study it is clear that during the early stages of regeneration viz. wound healing, preblastemic and blastemic periods, only the wound epithelium contains SDH. As differentiation commences the enzyme gradually appears first in the stratum germinativum, then the muscle fibres and the ependymal tube. In the fully regenerated tail the SDH activity was almost similar to that in the normal tail in its distribution and intensity.