

CHAPTER 7

HISTOCHEMICAL LOCALIZATION OF CHOLINESTERASE IN THE
NORMAL AND REGENERATING TAIL OF THE SCINCID LIZARD,MABUYA CARINATA

Since many years, the relationship between nervous tissues and regeneration had drawn the attention of a number of workers. As a result of studies on this aspect it is now known that nerve fibres are important for regeneration because of the action they exert on restoration of the lost parts. A series of investigations by Singer (1946, 1947 and 1952) on urodele amphibians have lead him to evolve the concept that a threshold number of nerve fibres is needed for the restoration of the forelimb. The importance of nerves even in the formation of blastema in the regenerating limb of urodele has been stressed (Butler and Schotté, 1941, 1949; Schotté and Butler, 1944). Further, the role of the number of nerves, in relation to the surrounding tissues at the levels of amputation of the limb, constituting a minimal threshold essential for the progress of regeneration has been brought out by Singer (1952) on urodeles and Van Stone (1955, 1957 and 1964) on anurans.

In an attempt to explore the trophic action of nerves on regeneration, nerve mediators such as acetylcholine, sympathin and others have been investigated (Le Camp, 1954; Schotté, 1926; Singer, 1959; Singer et al., 1960 and Taban, 1955). Acetylcholine has been studied biochemically and histochemically in the regenerating forelimb of the adult newt by Singer et al. (1960). Acetylcholinesterase (AChE) readily inactivates transmission of impulses by breaking the acetylcholine(ACh) released at neuroneural and myoneural junctions (Nachmansohn, 1946). Acetylcholinesterase differs from the other cholinesterase (Butyrylcholinesterase) in substrate specificity and the higher rate of hydrolysis of acetylcholine esters (Oosterbaan and Jansz, 1965).

The available literature todate regarding nervous participation in the lizard, tail regeneration are mainly due to the intensive studies of Kamrin and Singer (1955) on Lygosoma laterale; Simpson (1964, 1965) and Cox (1969b) on Anolis carolinensis and Shah and Chakko (1971) on Hemidactylus flaviviridis. The present histochemical study on the localization of cholinesterases; Acetylcholinesterase (AChE) and Butyrylcholinesterase(BuChE) was undertaken in this wake not only for an additional

knowledge on the role of nervous and nerve mediators during regeneration in general of vertebrate appendages but also specifically as a part of the elaborate biochemical investigations undertaken in this laboratory on the process of tail regeneration in the tail of the Scincid lizard, Mabuya carinata.

MATERIAL AND METHODS

Adult lizards obtained from the local animal supplier were maintained in the laboratory on a diet of young insects. The autotomy was induced by pinching off the tails about 4 centimeters from the vent. The autotomized tails were blotted to remove blood and tissue fluids and fixed on a microtome chuck of a cryostat maintained at -20°C . Sections of 18μ thickness were cut and fixed in chilled 10% formol-saline (Gurr, 1956) for 2-3 hours at 4°C and then washed thoroughly in distilled water. The method of Koelle and Friedenwald (1949) as modified by Coupland and Holmes (1957) was employed for the histochemical studies of cholinesterases. Acetylthiocholine Iodide and Butyrylthiocholine Iodide (Sigma Chemical Company, U.S.A.) were used as the respective substrates for the two esterases;

Acetylcholinesterase (AChE) and Butyrylcholinesterase (BuChE). The sections were incubated at room temperature (29-32°C) in an incubation medium maintained at a pH between 5.6 - 6; the incubation time ranging from 16-24 hours.

The control sections were treated with 3×10^{-5} M solution of eserine sulphate at room temperature for 30 minutes before incubation. After incubation, both the sample and control sections were thoroughly washed with distilled water, then treated with a dilute solution of yellow ammonium sulphide, washed again in distilled water, dehydrated through alcohol grades and mounted in canada balsam.

OBSERVATIONS

NORMAL TAIL

Of the various normal tail components, only skin, muscles and nerve cord responded for both the cholinesterases though with different intensities. The nerve endings at the skin region responded towards both the cholinesterases. Though, the caudal muscles revealed a very high intensity of both AChE and BuChE the former

tended to be higher than the latter. The localization was exclusively at the myoneural junctions (endplates) and they were of the 'en plaque' type (Kruger, 1958, 1960; Ginsborg and Mackay, 1960) (Fig. 1). An intense activity of both the cholinesterases was observable in the spinal cord.

REGENERATING TAIL

During the early phases of regeneration i.e., wound healing (Fig.2), preblastema and blastema (Fig.3), both the cholinesterases were noted to be negligible in all the cells of the regenerate. Only the injured regions of the spinal cord showed a slight activity of cholinesterases.

The cholinesterases made their appearance only during the differentiation phase of the regenerating tail. Amongst the various differentiating tissues, the muscles showed an intense response towards both the cholinesterases (Fig.4). Along with myogenesis, with the transformation of myoblasts into myocytes and later the myofibres, the activities of the two cholinesterases also registered a parallel increase. The localization of the enzyme was confined to sarcoplasm

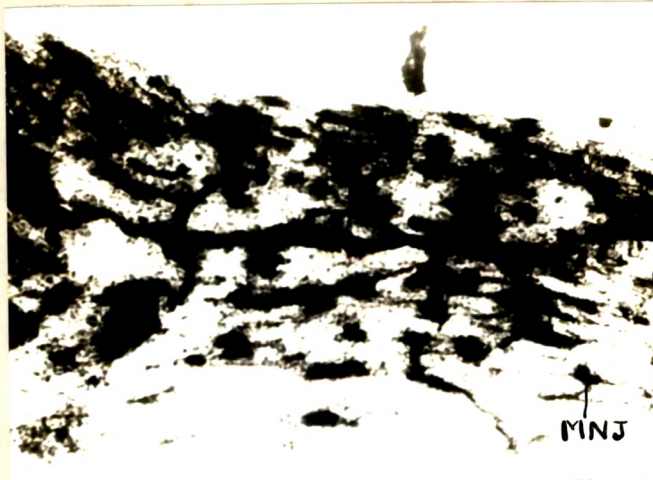
EXPLANATIONS FOR FIGURES

Fig. 1. Photomicrograph of the normal caudal muscles exhibiting acetylcholinesterase activity in the 'en plaque' type of myo-neural junction.

Fig. 2. Longitudinal section of the tail regenerate at wound healing phase. Note the negligible content of the enzyme in the wound epithelium.

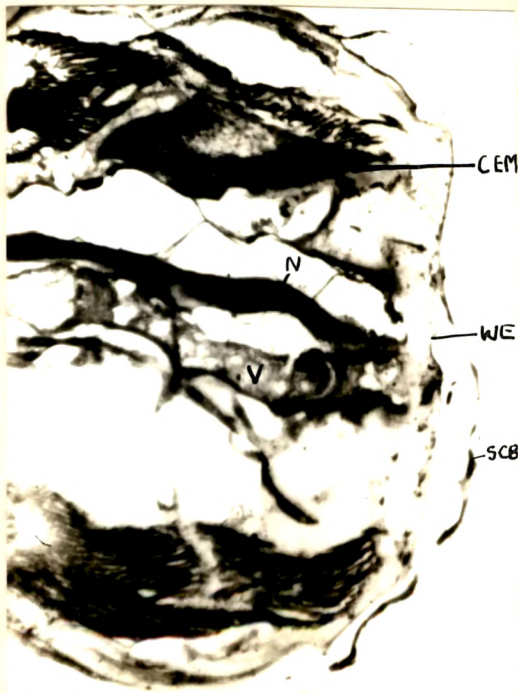
ABBREVIATIONS

CEM - Cut end of the muscle
MNJ - Myoneural junction
N - Nerve cord
SCB - Scab
V - Vertebra
WE - Wound epithelium



200 μ

1



2 mm

2

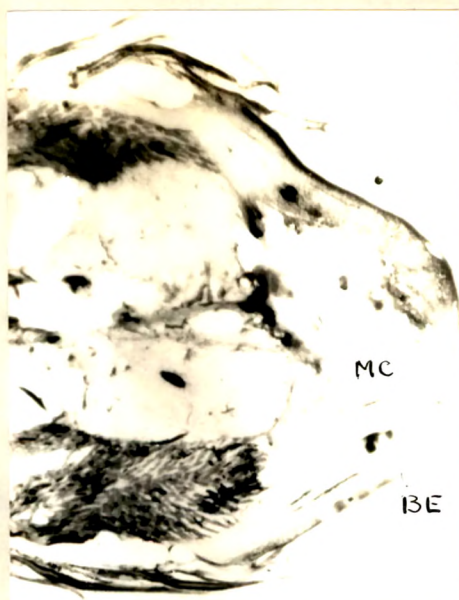
EXPLANATIONS FOR FIGURES

Fig. 3. Longitudinal section of the blastema revealing the negligible acetylcholinesterase activity in the mesenchymal cells and the blastemic epithelium.

Fig. 4. Longitudinal section of the tail regenerate during the differentiation phase revealing very high activity of AChE in the differentiating muscle and slight activity in the differentiating scales.

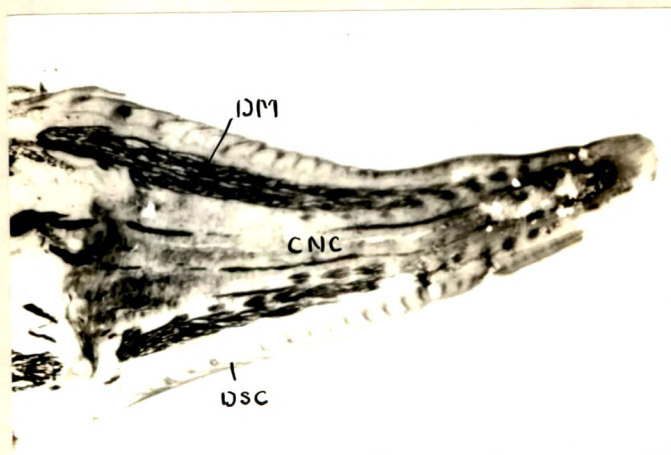
ABBREVIATIONS

BE	-	Blastemic epithelium
CNC	-	Cartilagenous neural canal
DM	-	Differentiating muscle
DSC	-	Differentiating scale
MC	-	Mesenchymal cells



2 mm

3



2 mm

4

during myogenesis. Excepting for the epidermal region of the skin which showed a low level of activity in comparison to the normal skin; all the other differentiating tissues failed to show any trace of cholinesterases. Nevertheless, the ependyma became positive towards both the enzymes with the progression of differentiation.

Through growth phase towards the fully regenerated condition, in the muscles there was a gradual shift in the localization of the enzyme reactivity from sarcoplasm to myoneural junctions (endplates). However, in the fully regenerated tail, the muscles presented a reduced intensity in comparison to the normal tail muscles. But the ependyma though showed a decreased level in comparison to the differentiation phase, depicted a level of cholinesterases very much equal to the one observed in the nerve cord of the normal tail.

DISCUSSION

The present investigation on cholinesterases has revealed a higher intensity of AChE in comparison to BuChE in the caudal muscles of the tail of Mabuya carinata localized chiefly at the myoneural junctions.

Similar high intensities of AChE in the limb muscles of Triturus viridescens and Diemictylus viridescens have also been recorded by Singer et al. (1960) and Schmidt and Norman (1965) respectively. But at the same time Chinoy and George (1965, 1966) suggested that acetylcholinesterase is characteristic of active, tonic and narrower red muscles whereas butyrylcholinesterase is associated with the tetanic and quick contracting white muscles of the pigeon pectoralis. Unlike the pigeon pectoralis, the caudal muscles of Mabuya carinata though characterised as the white and quick contracting tetanous type are more AChE active rather than BuChE. In this light, the present disparity seems to underscore either of a specialized nature or more probably the incompleteness of the evolutionary differentiation at biochemical level, of the reptilian muscles.

A noninvolvement of neurotrophic agents either in the initiation of regeneration or formation of early regenerates amongst the lizards becomes further emphasized by the herein observed slight activities of both the cholinesterases at the injured regions

of the nerve cord and the negligible activities during the wound healing, preblastemic and blastemic phases as had also been observed by Singer et al., (1960) and Shah and Chakko (1971) in their studies on Triturus viridescense and Hemidactylus flaviviridis respectively.

A gradual increase in the activities of both the cholinesterases observed during the differentiation phase in general may be correlated with the functional differentiation of tissues during the regeneration process. An increased activity of these enzymes during myogenesis is well supported by the studies of Verga et al., (1957) who also reported increased levels of cholinesterase activity correlated with the increase in the myosine cholinesterase level in developing muscles. The sarcoplasmic localization of cholinesterases reported in the developing muscles of Mabuya carinata is also in conformity with the earlier work of Gerebtzoff et al., (1954) who also reported a higher sarcoplasmic enzyme localization in prenatal stages and which decreased towards the end of incubation periods. Similar instances were also reported by Chinoy and George (1966) during the embryonic development

of the pigeon pectoralis muscles. A close association of acetylcholinesterase with myogenesis in regenerating system as well as in the process of differentiation during the development of various embryos were reported by Sawyer (1955) and Boell (1948,1955). These observations lead to surmise that the neurotrophic agents do not have a role in the initiation of the process of regeneration as such, they might on the contrary be of some significance in the process of histodifferentiation of tissues especially muscles.

The essential difference with the termination of the histodifferentiative mechanism seems to be a gradual decrease in the activities of both the cholinesterases through the growth phase. It is of interest that Simpson (1964) definitely showed that the ependyma is responsible for the initiation of tail regeneration in Lygosoma laterale. Coupled to this the present observations of increased cholinesterases in the tail ependyma of Mabuya carinata during the late differentiation phase could be indicative of a possible neurochemical mechanism either in the termination of differentiation process and or in the onset of growth process, though such a mechanism is not apparent in the initiation and during the early phases of regeneration as noted above.