CHAPTER 9

HISTOCHEMICAL LOCALIZATION OF CHOLINESTERASES IN THE NORMAL AND REGENERATING TAIL OF THE HOUSE LIZARD, HEMIDACTYLUS FLAVIVIRIDIS

It is known that the nerve supply plays an important role in initiating and maintaining regeneration in vertebrates (Rose, 1948; Singer, 1952,1956,1962). If the nerve supply towards the regenerating part is reduced below a certain threshold value, then the regeneration ceases to occur (Singer, 1962; Van Stone, 1964). Schotté and Butler (1941) have shown that in amphibians the initiating capacity of the nerves upon regeneration is more profound in larval forms than in the adults. On the other hand, Yntema (1959) had reported the occurrence of regeneration without nerve supply under certain circumstances. Recently, Singer (1961), Simpson (1961) and Kudokotsev (1962) induced regeneration of limbs in lizards by increasing the nerve supply. The trophic influence of the nerve has been suggested to be chemical in nature (Singer, 1959). But the responsible neurochemical agents are not yet known. The effects of acetylcholine, atropin and sympathin on regeneration have been studied in order to understand the trophic effect of nerves on regeneration (Singer, 1959, 1960). The present histochemical study on the acetyl- and butyrylcholinesterase activity in the tissues of the normal and regenerating tail

of the house lizard, Hemidactylus flaviviridis, was carried out with a view to obtain more information on the trophic influence of cholinesterases on regeneration in reptiles.

MATERIALS AND METHODS

The normal and the regenerating tails with atleast one or two segments of the original tail stump were cut and fixed in chilled 10% formol saline (Gurr, 1956) for 4 to 6 hours at 4°C. After fixation the tissues were washed thoroughly with distilled water and sectioned at 10 to 15 μ on a freezing microtome. The sections were incubated at 37°C at a pH of 5.6 to 6 in order to demonstrate the acetyl- and butyrylcholinesterase activities separately by the method of Koelle and Friedenwald (1949) as modified by Coupland and Holmes (1957). Acetylthiocholine-iodide and butyrylthiocholine-iodide (Sigma Chemical Co., U.S.A.) were used as the respective substrates for the two esterases. The incubation time ranged from 19 to 23 hours. The control sections were treated with a 3×10^{-5}M solution of eserine sulphate at room temperature for 30 minutes before incubation. After incubation the sample and control sections were thoroughly washed with distilled water, treated with a dilute solution of yellow ammonium sulphide, washed again with distilled

water, dehydrated and mounted in canada balsam.

OBSERVATIONS

NORMAL TAIL:

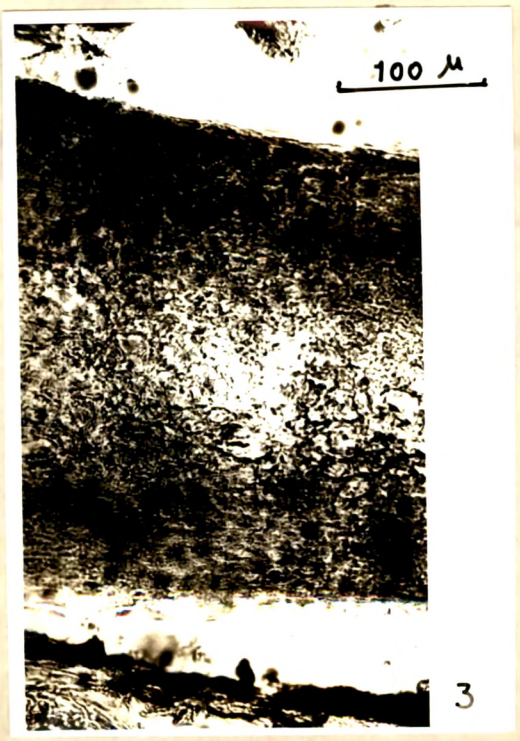
Except for some nerve innervations, the skin, adipose tissue and the vertebral column of the caudal region did not show either acetyl- or butyrylcholinesterase activity. On the other hand, the muscle fibres revealed both cholinesterases. The localization was found to be in the sarcoplasm, myoneural and myotendinous junctions (Figs. 1 & 2). The myoneural endings were of the "en plaque" type. All the fibres irrespective of broad or narrow type stained more intensely for acetylcholinesterase than for butyrylcholinesterase. An intense acetyl- and butyrylcholinesterase activity was observed in the spinal cord (Fig. 3).

REGENERATING TAIL:

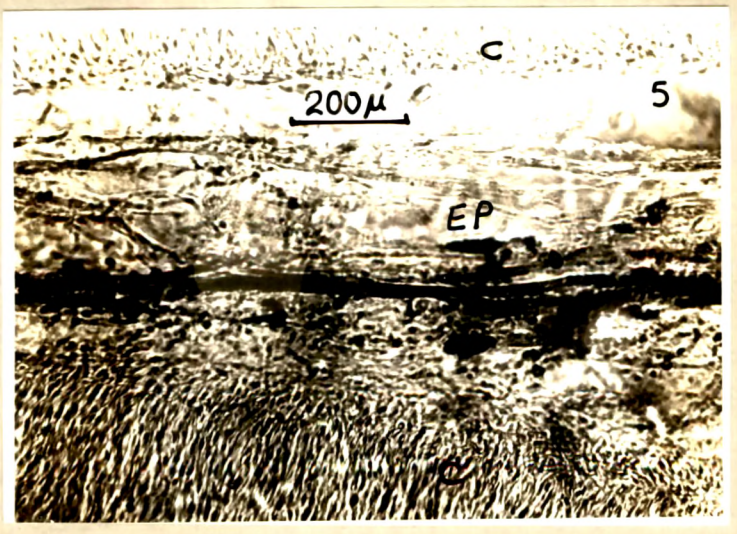
In the early phase of regeneration, the cholinesterases were absent in all the tissues. It was only when the differentiation started that the newly differentiated mononuclear myoblasts and the cells of the ependyma revealed the acetyl- and butyrylcholinesterases (Figs. 4 & 5). However, the latter appeared slighly later than the former in the myofibres. The other tissues viz. skin, adipose tissue and the cartilagenous neural canal did not give any reaction for these esterases.


50 μ
1


50 μ
2


100 μ
3


100 µ
4


C
200µ
5
EP

EXPLANATIONS FOR FIGURES

Fig. 1. Demonstration of cholinesterases at the "en plaque" type of end plate in the caudal muscle fibres of the normal tail.

Fig. 2. Localization of cholinesterases at the myotendinous junctions of the caudal muscle of the normal tail.

Fig. 3. L.S. of spinal cord showing cholinesterases

Fig. 4. Localization of cholinesterases in the mononuclear myoblasts.

Fig. 5. L.S. of regenerate passing through the ependyma showing cholinesterases.

ABBREVIATIONS

C - Chondrocytes

EP - Ependyma

Only the nerves and the nerve innervations in these tissues showed the enzymatic activity.

During myogenesis in the regenerating tail, cholinesterase activity was localized mainly in the mononuclear myoblasts. The intensity of the enzyme increased as the myoblasts transformed into myocytes and later into myofibres. However, in the fully regenerated muscle fibres the enzyme activity never reached the original level noticed in the normal tail. The localization of these enzymes became more specific at the "en plaque" myoneural regions when the myofibres began to mature.

The activity of the acetylcholinesterase was more than butyrylcholinesterase in all the tissues of the normal and regenerating tail of the house lizard.

DISCUSSION

The histochemical studies on the acetyl- and butyrylcholinesterases in the normal tissues of the tail of the house lizard, H. flaviviridis revealed the presence of both the enzymes in the muscles and the spinal cord. However, in the other tissues, the nerves alone showed the enzyme activity. Both cholinesterases were found to be localized in the sarcoplasm, myoneural and myotendinous

junctions of muscles. In all the muscle fibres the acetylcholinesterase activity was found to be more intense than butyrylcholinesterase. Similarly, Singer et al. (1960) and Schmidt and Norman (1965) reported intense cholinesterase activity in the striated muscles of the forelimb of Triturus and in the limb of adult newt, Diemictylus viridescens respectively. Chinoy and George (1965, 1966) also reported that the cholinesterases were located in the sarcoplasm and at myoneural junctions of the pectoral muscles of a variety of vertebrates and the developing pigeon pectoralis. Further, they have suggested that in an active tonic muscle, the activity of acetylcholinesterase was more than that of butyrylcholinesterase. Thus, the red tonic muscle fibres of the pigeon breast muscle and also such fibres of other actively flying birds possessed more acetylcholinesterase and the white tetanic fibres more of butyrylcholinesterase (Chinoy and George, 1965). The histochemical observations on the muscle of the lizard tail showed that most of the fibres are of the tetanic type. The narrow and intermediate fibre types were confined to a narrow band towards the peripheral region of the fasciculi. The observations, that all the fibres irrespective of broad or narrow type, showed a higher activity of acetylcholinesterase in comparison to butyrylcholinesterase, does not agree with the findings

of Chinoy and George (1965). It may well be that in the lizard tail muscle, the distinction of the fibres into broad tetanic, adapted for anaerobic metabolism and narrow tonic, adapted for aerobic metabolism are not as clear cut as in the pectoral muscle of pigeon.

The grey matter in the spinal cord of the normal tail showed intense activity of both the enzymes as compared to the white matter. There was no change in the activity of these enzymes at the cut end of the spinal cord in the tail stump during the wound healing, pre-blastemic or blastemic phases of regeneration. Only the injured cells which were disintegrating showed poor enzyme activity. The just differentiating ependyma also showed a negligible activity of cholinesterases. As the differentiation progressed and the ependyma was formed, the enzyme activity gradually increased but even in the fully grown ependyma, the enzyme activity never reached the original level present in the spinal cord. This suggests that in the initial stages the cholinesterases are not of much importance as far as the initiation of regeneration is concerned. Perhaps in the later stages of regeneration when the ependyma acquires a fairly perceptible enzyme activity, these esterases may have a hitherto unknown role to play.

Acetyl- and butyrylcholinesterases, both appeared very late in regeneration i.e. during the differentiation period at the onset of myogenesis. The early regeneration period viz. wound healing, preblastemic and blastemic phases were negative to cholinesterases. The enzymes appeared as soon as the myoblast differentiation took place. The localization was confined only to the sarcoplasm. The specific localisation in the myoneural and myotendinous junctions was observed later when the fibres attained growth and maturity. A similar condition has been described during the fore limb regeneration of the newt, Triturus (Singer et al., 1960). They found that the enzyme was subnormal for 10 days after amputation, increased with differentiation of paddle form regenerate and followed by a decline towards the normal enzyme activity with maturation of the digit bearing limbs. The present study in the regenerating tail agrees with the above fluctuations of the enzyme reported by Singer et al. (1960). First the enzyme activity appeared subnormal as the wound healing, preblastemic and the blastemic stages did not show any enzymatic activity. Only during the differentiation period, a sarcoplasmic enzyme response was noted. This increase in the enzyme could be correlated with the differentiation period. Later, there was a decrease in the enzymatic levels towards the time of maturity and the growth of the

muscle fibres, because this was the period when a reduction in the sarcoplasmic cholinesterases was noted and the enzymes became more prominent in the myoneural and myotendinous junctions. Varga et al. (1957) observed that increase in the total cholinesterase activity was due to a considerable increase in the myosincholinesterase level which was higher in early stages of the development than in the adult. The sarcoplasmic cholinesterase activity was reported to be higher before hatching or in prenatal stages which decreased towards the end of the incubation period (Gerebtzoff et al. 1954). Similar observations were reported by Chinoy and George (1966) on the embryonic development of the pigeon pectoral muscle. These observations are in confirmity with the results of the present investigation.

As a general feature, in the normal and the regenerated tails, it was found that the acetylcholinesterase activity always exceeded that of the butyrylcholinesterase. As mentioned earlier, in the early periods of regeneration hardly any activity of these enzymes was noticeable so it confirms the findings of Singer (1959). Singer et al. (1960) reported that cholinesteráses do not intiate or stimulate the regeneration as a neurotropic agent.