CHAPTER 3

CHOLINESTERASES IN THE PIGEON PECTORALIS DURING DISUSE ATROPHY

In the recent years considerable amount of work has been done on the histochemistry, biochemistry and physiology of cholinesterases in various tissues particularly in muscle and nerve. The intense activity of these enzymes is a characteristic feature of motor nerve endings. Based on the nature of the nerve endings, fibres of the vertebrate skeletal muscle were classified as "Fibrillenstruktur" and "Felderstruktur" (Krüger and Gunther, 1958; Krüger, 1960; Ginsborg, and Mackay, 1960). The former ones were found to be focally innervated by "en plaque" nerve endings having acetyl cholinesterase and the latter exclusively by several closely arranged neuromuscular junctions of "en grappe" type containing butyryl cholinesterase. Thus the two basic type of muscle fibres the broad, white tetanic, and the red, narrow tonic were believed to have the "enplaque" and the "en grappe" type of innervations respectively (Ginsborg and Mackay, 1960). But these observations do not hold good for a mixed muscle like the pigeon pectoralis, where the neuromuscular junctions of the broad, white, and the narrow, red fibres were found to be alike, and of only the "en plaque" type (Chinoy and George, 1965).

Moreover, this was also found to be true for a number of vertebrate pectoral muscles and both the esterases were present at these nerve endings. Variations in their concentrations however, did occur depending on the functional adaptation of the muscle as observed by the above authors.

In the normal pigeon breast muscle, the non-specific cholinesterases (BuChE) was higher than the specific (AChE) in the broad, white fibres (Chinoy and George, 1965). But the enzyme concentration, particularly that of AChE increased, when the muscle was electrically stimulated, and by the low concentrations of sodium and potassium (Chinoy, 1965).

Since the increase in the cholinesterase level is correlated with the functional activity of the muscle, a low concentration of the enzyme should be expected when the muscle is put to disuse. Thus the earlier workers (Brizin and Majcen-Tkacev, 1963) obtained a decrease in cholinesterase (ChE) activity at the myoneural junction during denervation atrophy of the muscle, as well as absence of nerve endings from atrophied fibres in certain cases of muscular atrophy (Cöers, 1957). In dystrophies on the other hand the neuromuscular junctions seemed to remain unimpaired. But it is not judicious to generalize on the basis of the above mentioned observations since there is considerable disparity in ChE activity in the

different types of muscle diseases. Thus in some atrophies especially in dystrophia myotonia the end plates remained intact and no perceptible decrease in ChE at the nerve endings of the diseased muscle was discernible (Beckett and Bourne, 1957).

In the light of these observations it was thought worthwhile to study the changes in ChE activity in the pigeon pectoralis muscle under different stages of disuse atrophy.

Material and Method

Well fed laboratory bred pigeons weighing between 300-320 g. were used. Their wings were placed in a plaster cast as described in chapter 1. The birds were sacrificed on the 7th, 14th, 21st, and 30th day after inducing disuse atrophy. They were decapitated and in each case a piece of the muscle from the central portion of the pectoralis was removed and blotted completely free of blood. The excised tissue was immediately frozen on the stage of a freezing microtome and longitudinal sections about 20 μ were cut. Sections were fixed in chilled 10% formol saline (Gurr, 1956) for about 1-3 hours at 4°C., washed thoroughly with redistilled water and incubated separately to demonstrate both AChE and BuChE activity. The method employed was that of Koelle and Friedenwald (1949) as modified by Coupland and Holmes (1957).

The control sections were treated prior to incubation in a solution of $3x10^{-5}$ M. eserine sulphate for 1 hour at room temperature in order to destroy the esterases and then incubated along with the samples.

For each stage of atrophy studied, the optimum incubation time required to demonstrate the maximum ChE activity was determined by treating the sections with yellow ammonium sulphide at regular intervals of 1 hour. The minimum time required to obtain the maximum activity was taken to be the optimum time. After treatment with yellow ammonium sulphide the sections were washed thoroughly, dehydrated and mounted in canada balsam.

Results

The results obtained are presented in table 1 and in figs. 1-6. The most significant feature observed was the increased activity of both cholinesterases on the 7th day after immobilization of the wings. The activity of AChE was clearly visible at the motor end plates within four to six hours of incubation and that of BuChE by six to eight hours. After the 7th day of disuse atrophy the levels of both the enzymes seemed to decline and were found to be fluctuating (Table 1). Thus in some cases the activity of the 21st day was slightly higher than that of the 14th day as seen from the lesser incubation time required to obtain the maximum reaction. These fluctuations in the enzymatic levels might be due to the individual variations. However, by the 30th day of atrophy the enzyme

TABLE 1

Cholinesterase activity in the pigeon pectoralis muscle under disuse atrophy

	Days of disuse atrophy									
	Acetylcholinesterase					Butyrylcholinesterase				
Incubation time in hr.	Normal	7	14	21	30	Normal	7	14	21	30
4	-	±	±	±	±	-	-	-	-	-
6	-	++	±	±	±	±	±	-	-	-
8	-	+++	±	±	±	-	++	±	±	-
10	±	+++	±	+	±	-	+++	+	+	-
15	±	+++	++	++	+	+	+++	++	++	+
20	++	+++	+++	+++	++	+++	+++	++	++	+
24	+++	+++	+++	+++	+++	+++	+++	+++	++	++

Maximum activity +++ ., Medium activity ++ , Minimum activity + , Negligible ± , Nil - .

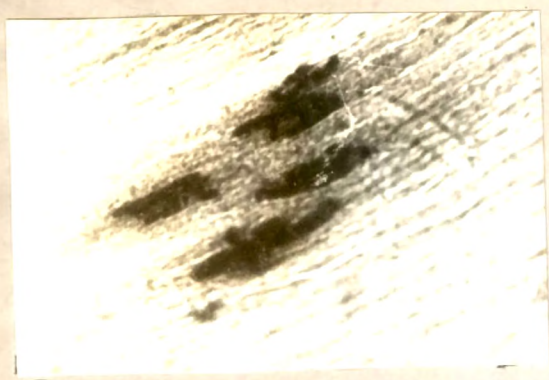

Fig. 1. Longitudinal section (L.S.) of normal pigeon pectoralis muscle stained to demonstrate acetylcholine esterase activity. Incubation time, 24 hr. X 256.
Fig. 2. L.S. of normal pigeon pectoralis stained for butyryl-cholinesterase activity . Incubation time 20 hr. X 256.

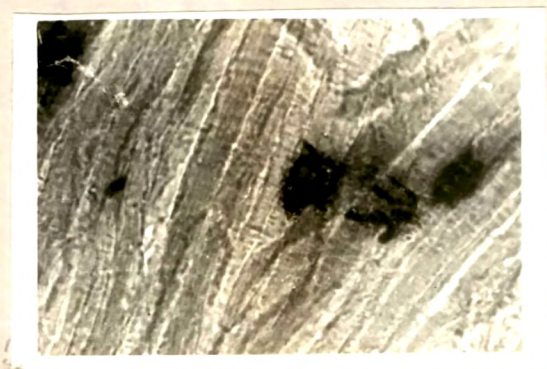

Fig. 3. L.S. of pigeon pectoralis muscle showing the localization of acetylcholinesterase activity after 7 days of disuse atrophy. Incubation time, 8 hrs. X 256.

Fig. 4. L.S. of pigeon pectoralis muscle showing the localization of butyrylcholinesterase activity after 7 days of disuse atrophy. Incubation time, 8 hr. X 256.

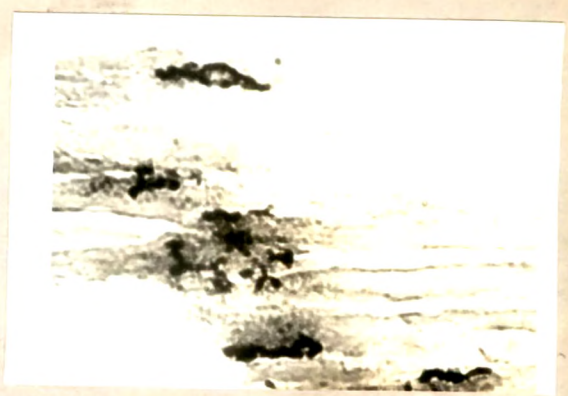

Fig. 5. L.S. of pigeon pectoralis muscle showing the localization of acetylcholinesterase activity after 30 days of disuse atrophy. Incubation time, 24 hr. X 256.
Fig. 6. Same as above but showing the activity of butyrylcholinesterase.

concentration was by and large similar to the normal, if not identical. It has also been observed that the levels of AChE were comparatively higher than that of BuChE throughout . In all the four stages investigated there was no alteration in the morphological structure of the neuromuscular junctions despite the significant variation in the enzyme activity at these sites.

Discussion

That cholinesterases are indispensable for normal neuromuscular transmissions is well established. It is also known that both the enzymes are located indiscriminately at every nerve ending (neuromuscular and myotendinous) and in the sarcoplasm of the slow and fast fibres of numerous vertebrate skeletal muscles (Gerebtzoff, 1959; Klinar and Župančič, 1962; Bokdawala, 1965; Chinoy and George, 1965; Joseph, 1967).Further, a higher level of AChE was demonstrated in the red fibres of the more active muscles such as trunk muscles of fishes (Bokdawala, 1965; Joseph, 1967),and the pectoral muscles of a number of vertebrates (Chinoy and George, 1965). The white muscles on the other hand, possessed a greater store of BuChE. The two esterases were shown to be influenced by factors such as electrical stimulation, electrolyte changes (Na^+ and K^+), starvation and growth (Chinoy, 1965). If on exercise, the ChE activity in the muscle increases it is logical to expect a depletion in the activity of the

enzyme when the muscle is put to disuse. But the result obtained in the present investigation were to the contrary.

Earlier studies on denervation atrophy and its effect on muscle cholinesterases by a number of workers (Brizin and Mjcen-Tkacev, 1965; Gerebʓtzoff and Vandersmission, 1965; Varga et al., 1957) had indicated changes in the enzyme activity but never the total inactivity of of the enzyme. This is indicative of the significance of motor innervation of a muscle. The presence of the intact nerve endings with increased ChE activity under disuse atrophy of the muscle clearly shows (1) the importance of nerve connections in the normal functioning of the muscle; and (2) that the enforced immobility of the muscle does not inhibit in any way, the impulse transmitting mechanism, because in spite of being in the plaster cast, a severe shivering action of the wings occurred. This might be to resist the state of enforced immobility and thus causing a mild increase in ChE activity.

The increase in the enzyme concentration observed during the early days of disuse atrophy could also be correlated with a corresponding increase of potassium in the muscle (Chapter 4). It has already been shown (Nachmansohn, 1940; Chinoy, 1965) that the monovalent cations sodium and potassium have a definite role in the enhancement

of ChE activity. So the possibility that the enhanced enzyme level is the result of increased potassium cannot be over ruled.

The plaster casted pigeons were observed to be in a condition of stress and also starvation despite the adequate supply of the normal diet. Starvation has been shown to cause a definite rise in ChE activity at the nerve endings (Chinoy, 1965). and the enzyme activity seen during the earlier period of disuse atrophy may also be due to this particular state of the animal.

In the later stages of atrophy however, the enzyme activity declined, though not gradually. Even after a period of 30 days it did not seem to attain the normal level. This suggests that disuse atrophy did have some effects upon the neuromuscular transmission, though not a significant one. Beckett and Bourne (1957a, 1957b, 1957c) also expressed doubt regarding the real significance of the slight variation in the ChE activity observed in the muscles of dystrophic patients. It is only in some neuromuscular diseases like myasthenia gravis, that a defect in the neuromuscular transmission has been observed.

The observations made in the present study therefore indicate that no serious impairment of the neuromuscular transmission occurs under the present conditions of disuse atrophy. The variation seen in the enzyme activity

seemed to be influenced directly or indirectly by several factors which are also to be taken into account in arriving at conclusions.