CHAPTER 4

CHOLINESTERASES IN THE DIAPHRAGM OF HUMAN FOETUS AND ADULT

It is well known that the diaphragm in a mammal starts functioning soon after parturition. The mammalian diaphragm is a skeletal muscle of the mixed variety having the red, white and intermediate types of fibres. These three types of fibres were shown in the various animals by various workers. Even in the adult human these three types of fibres can be easily distinguished with the help of SDH staining, as well as with the fat staining using Sudan Black as was demonstrated by Gauthier and Padykula (1966). In foetal diaphragm the two basic types of fibres can be clearly observed after staining the tissue sections with Sudan Black.

The red and white fibres of a mixed muscle not only differ in colour but also histochemically and morphologically in the nature of their neuromuscular junctions and the type of cholinesterases located at these sites. The white tetanic fibres are innervated by "en plaque" nerve-endings which contain predominantly Acetyl cholinesterase (AChE) and the red tonic fibres by the "en grappe" type having Butyryl cholinesterase (BuChE) (Krüger, 1952, 1958, 1960; Häggqvist, 1960; Zenker and Anzenbacher, 1964). Further Klinar and Župančič (1962) demonstrated both the types of cholinesterases at every

nerve-endings regardless of the type of fibres in which they were located. Recent investigations by Chinoy and George (1965) on the pectoralis major muscle of a variety of vertebrates showed that the nerve-endings on the two types of fibres (in all the animals studies), were of "en plaque" type. The red tonic fibres possessed more of acetyl cholinesterase while the white tetanic fibres possessed more of butyryl cholinesterase. Therefore acetyl cholinesterase is in greater amounts in muscles indulging in sustained activity. These observations have significant implications on the functional aspects of pectoralis.

The present histochemical investigation on cholinesterases was conducted on yet another important muscle of a mixed variety namely the diaphragm in order to study the morphological nature of the nerve-endings and their enzymic levels in the pre-natal, post-natal and adult human diaphragm.

MATERIALS AND METHODS

The diaphragm was collected from still born babies at different stages of development, and an adult. In most of the cases the babies were stored in the cold room for 1 to 6 hrs. The autopsy material was blotted well to remove blood and was spread on a clean filter paper. The three regions (dorsal, lateral and ventral as shown in Fig. 1 of Introduction) were then separated as described by George and Susheela (1961).

From each of the three regions, fresh frozen longitudinal sections (about 20 μ) were cut, and fixed in chilled 10% formol saline (Gurr, 1956) for 1 to 2 hrs. The sections were then rinsed thoroughly with distilled water and incubated at 37°C. in a medium (pH 5.6 to 6.0) prepared according to the modified method of Coupland and Holmes (1957). Sections of the three regions were incubated separately in order to demonstrate the two enzymes, namely the AChE and BuChE using acetyl thiocholineiodide and butyryl thiocholineiodide as the respective substrates. The enzyme activity was judged by the duration of the incubation period and the intensity of the enzyme reaction. The area of the contact surface was determined by measuring the diameter of the end-plates as suggested by Gerebtzoff (1959).

OBSERVATIONS

The results obtained are presented in Table 1.

(1) In spite of being a mixed muscle, the human diaphragm in all the cases investigated, had only the "en plaque" type of nerve-endings (Figs. 1 to 14).

(2) AChE and BuChE activities were both found to be present at the same nerve-endings.

TABLE 1

Cholinesterase activities in the human foetus and adult

No. of the case	Date of collection Age, Wt. at the time of Birth, Sex and the Cause of death	Activity as shown by the period of Incubation(in hours)						Intensity of the enzyme activity					
		Dorsal Region		Lateral Region		Ventral Region		Dorsal Region		Lateral Region		Ventral Region	
		AChE	BuChE	AChE	BuChE	AChE	BuChE	AChE	BuChE	AChE	BuChE	AChE	BuChE
1.	29th Dec. 1964, 8½ months, 2350 gms., Female.	14	15	18	18	Very low	Very low	++	++	+	+	-	-
2.	29th Dec. 1964, 8½ months, Wt. not taken, Male.	13	15	18	18	Very low	Very low	++	++	+	-	-	-
3.	30th Dec. 1964, 8½ months, 1900 gms., Male.	13	15	17	17	Very low	Very low	+++	++	+	+	-	-
4.	22nd Jan. 1965, 9 months, 2700 gms., Male, rupture of uterus.	13	14	12	14	14	14	+++	++	+++	++	++	++
5.	20th Mar. 1965, Full term, Wt. not taken, Female, forcep case.	14	16	13	16	15	16	++	+	+++	+	++	+
6.	20th March 1965, Full term, (Survived for a day after birth), Male, Wt. not taken.	14	15	13	14	14	15	++	++	+++	++	++	++
7.	11th March 1965, 20 years, An adult male, Accidental death.	14	16	12	16	16	16	++	+	+++	+	+	+

Note:- 12-13 hrs. high activity, +++. 14-15 hrs. moderate activity, ++. 16-18 hrs. low activity, +. Very low activity, -. (Could not be detected after prolonged incubation).

Contd....

Table 1 (Conta.)

No. of the case	Dorsal Region		Lateral Region		Ventral Region	
	AChE	BuChE	AChE	BuChE	AChE	BuChE
1.	Round 14.61	Activity is found to be diffused	Round or oblong 11.65	Round 14.00	-	-
2.	Round 16.12	Round 16.00	Round 11.88	Round 12.25	-	-
3.	Round 15.00	Round or slightly oblong 15.00	Round 11.75	Round or slightly oblong 12.50	-	
4.	Round or slightly oblong 18.00	Round or slightly oblong 16.40	Round or slightly oblong 21.60	Round or slightly oblong 17.17	Round or slightly oblong 18.00	Round or slightly oblong 15.12
5.	Round or oval 18.00	Round or oval 16.20	Round or oval 18.50	Round or oval 17.00	Round or oval 16.56	Round or oval 15.48
6.	Round or oval 17.28	Round or oval 15.80	Round or oval 18.36	Round or oval 14.00	Round or oval 14.40	Round or oval 14.00
7. Adult Human	Round 21.60 Much elongated 25.50 long and 10.50-14.50 broad.	Round or oval 23.20	Round 28.50 Much elongated 30.00 long and 14.00 broad.	Round or oval 25.00	Round or oval 21.50	Round or oval 20.50

(3) AChE activity was found to be higher than BuChE in majority of the cases studied.

(4) In foetuses of 8 to 8½ months pre-natal development, the dorsal region of the diaphragm showed the highest activity of both AChE and BuChE, that of AChE being higher than BuChE. The lateral region revealed the lower activity as compared to the dorsal region, whereas in ventral region the activity of both the cholinesterases was found to be very low or negligible (it could not be clearly observed even after 24 hrs. of incubation).

In the full-term, still born cases, the activity of AChE was again high as compared to that of BuChE in all the three regions. The lateral region exhibited the highest activity of both the cholinesterases, and the ventral region the least.

In the case of the adult (20 years old) the activity of both the cholinesterases was found to be nearly the same as in the full-term babies (Table 1).

(5) The end-plates in diaphragm of adult human were found to be very much enlarged as compared to the full-term babies.

In the first three cases the nerve-endings of the dorsal region were the largest (14-18 μ in diameter) as compared to

the other two regions (11-16 μ in diameter in the lateral and the ventral regions) and these nerve-endings were round or slightly oblong in shape. In all the other cases, the neuromuscular junctions of the lateral region were the largest in diameter (Table 1) than those of either the dorsal or the ventral region. These nerve-endings were found to be round or oval in the case no. 4, 5 and 6; while they were found to be much elongated in the adult (Case no. 7). From Table 1, it is clear that the neuromuscular junctions gradually increased in dimensions and altered in their shape with the development, longest being observed in the adult.

(6) In all the cases studied the end-plates of the ventral were found to be smaller than the other two regions of the diaphragm.

DISCUSSION

The diaphragm is a mixed muscle, where the distribution of the two types of fibres does not show any definite pattern of distribution and appears to be at random. The two types of muscle fibres were clearly observed in the diaphragm of the human foetus when stained for the histochemical demonstration of SDH (Succinate dehydrogenase) and fat. In the diaphragm of the adult human also the three types of fibres namely the red, narrow; white, broad; and the intermediate fibres were

demonstrated by Gauthier and Padykula (1966) with the help of the histochemical staining for fat with Sudan Black. The regional differences of the three different regions of the rat diaphragm namely the dorsal, lateral and ventral were studied by George and Susheela (1961). They have shown that the lateral region is specialized for carbohydrate metabolism as well as capable of oxidizing fatty acids more rapidly than the two other regions. Beck and Baxter (1960) showed that amongst the three regions the lateral has the major blood supply. From previous studies (Chapter 3) on the histochemical localization of cholinesterases in the diaphragm of different mammals, the lateral region was found to be the most important region taking an active part in the respiratory movements of the organ, because of the fact that the lateral region showed the highest activity of these enzymes as compared to the other two regions. Generally in all the regions the AChE activity was found to be higher as compared to that of BuChE as it was described by Smith et al. (1963). They showed that about 90% of the cholinesterase activity of the skeletal muscle is due to AChE while only 10% is due to BuChE.

The present investigation shows that the foetal diaphragm of about 8 to 8½ months of pre-natal development is inactive and does not show much activity of both the

cholinesterases. In these studies it was also observed that during this stage of pre-natal development even the end-plates are not well differentiated. In the dorsal region the activity of these enzymes is found to be higher as compared to the lateral, while the ventral shows very low activity. The reason may be that the lateral and the ventral regions might not have approached the functional differentiation. Nachmansohn (1940b) concluded that the concentration of the cholinesterases is more closely related to the functional differentiation than to the histochemical formation, from his studies of the brain and the spinal cord of the sheep embryos.

The foetal diaphragm of about 9 months of pre-natal development clearly shows that the lateral region has high activity of both the cholinesterases and bigger end-plates as compared to the other two regions. The activity of these esterases have increased, which can be correlated with the functional development of the muscle as was described by Hooker (1936) from his studies of cholinesterases in back and shoulder muscles. From the results given in Table 1, it can be clearly observed that the foetus of 9 months, a full-term baby and a child about one day old show more or less same activity of these esterases. Even in the adult human diaphragm the similar activity was observed which shows that at about 9 months of pre-natal development the diaphragm

must have completed its functional development. Though the activity of the cholinesterases in the adult as well as in the full grown and new born babies is similar as it appears from the period of incubation for the histochemical demonstration of the enzymes, a marked increase in the size of the end-plates was observed as it appears from the diameter of the end-plates. Such an increase in the size of the end-plates was also reported by Cuajunco (1942). The later studies made by Kuffer and Koelle (1951) on the formation of the motor end-plates of the developing fore limb musculature of the albino rats showed that there is no significant decrease in the cholinesterase activity during the development. However, the quantitative study of cholinesterase activity per miligram wet weight of the tissue was found to be less in the adult than at the birth, was supposed to be probably due to the decrease in the ratio of the end-plate volume with the muscle volume (Nachmansohn, 1939).

Thus from the present work it can be concluded that the general pattern of the adult end-plates and the cholinesterase activity are laid down at about 9 months of pre-natal development. During post-natal development the level of both the enzyme activities remain unchanged while the size of the end-plates gradually increases along with an increase in the size of the muscle fibres.

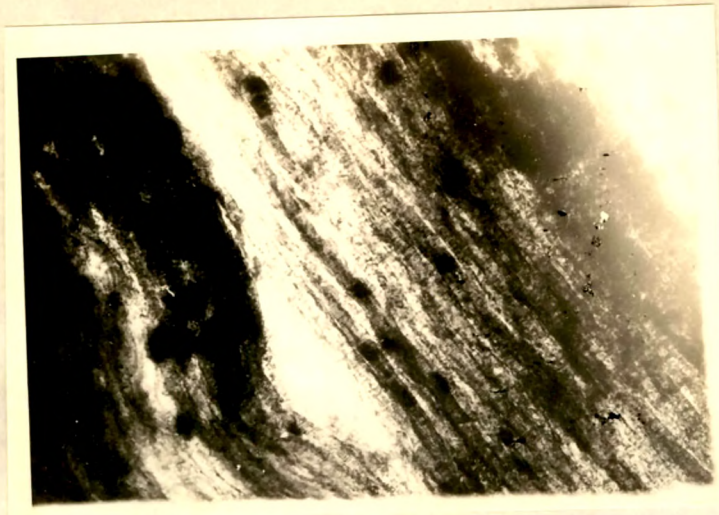

Fig. 1

Longitudinal section showing the histochemical localization of AChE in the dorsal region of the diaphragm of human foetus at 8½ months of pre-natal development. The nerve-endings are of "en plaque" type. 128 X

Fig. 2

Longitudinal section showing the histochemical localization of BuChE in the dorsal region of the diaphragm of human foetus at 8½ months of pre-natal development. The nerve-endings are of "en plaque" type. 128 X

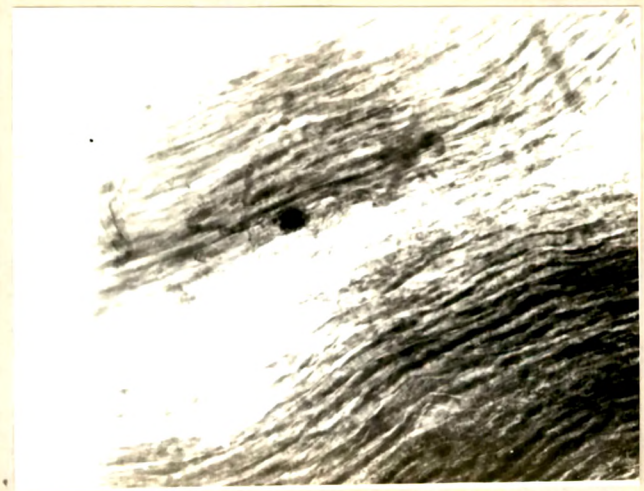

Fig. 3

Longitudinal section showing the histochemical localization of AChE in the lateral region of the diaphragm of human foetus at 8½ months of pre-natal development. The nerve-endings are of "en plaque" type. 128 X

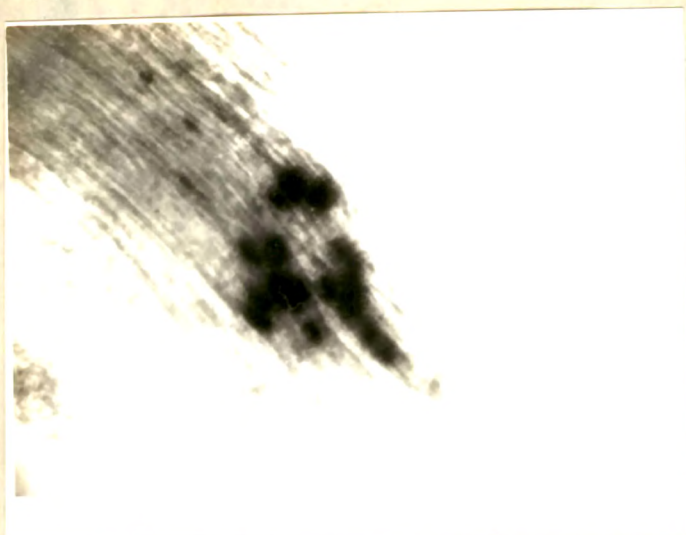

Fig. 4

Longitudianl section showing the histochemical localization of BuChE in the lateral region of the diaphragm of human foetus at 8½ months of pre-natal development. The nerve-endings are of "en plaque" type. 128 X

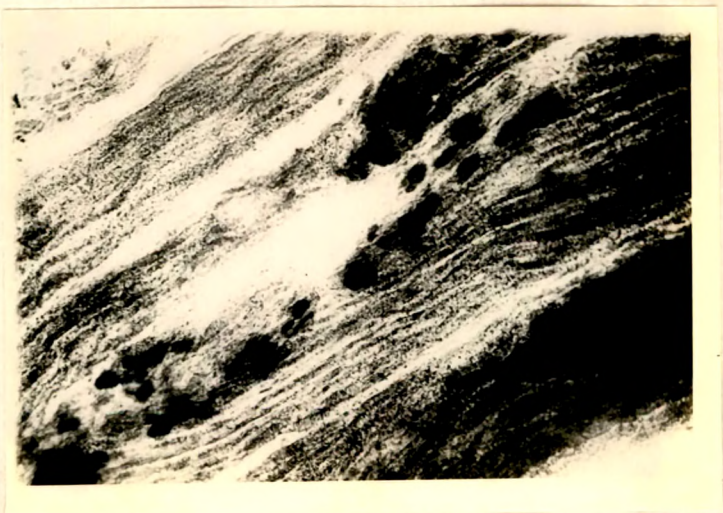

Fig. 5

Longitudinal section showing the histochemical localization of AChE in the dorsal region of the diaphragm of human foetus at 9 months of development. The nerve-endings are of "en plaque" type. 128 X.

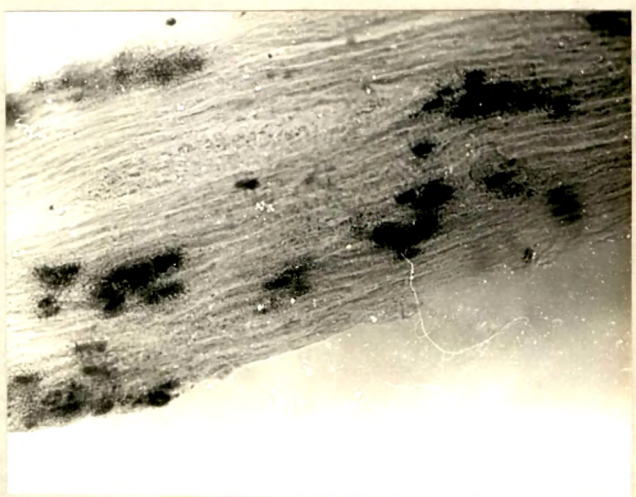

Fig. 6

Longitudinal section showing the histochemical localization of BuChE in the dorsal region of the diaphragm of human foetus at 9 months of pre-natal development. The nerve-endings are of "en plaque" type. 128 X

Fig. 7

Longitudinal section showing the histochemical localization of AChE in lateral region of the diaphragm of human foetus at 9 months of pre-natal development. The nerve-endings are of "en plaque" type. 128 X

Fig. 8

Longitudinal section showing the histochemical localization of BuChE in lateral region of the diaphragm of human foetus at 9 months of pre-natal development. The nerve-endings are of "en plaque" type. 128 X

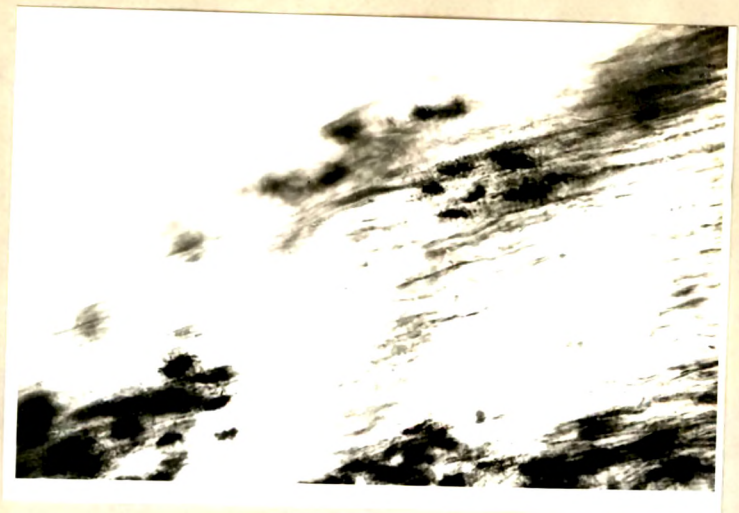

Fig. 9

Longitudinal section showing the histochemical localization of AChE in the ventral region of the diaphragm of human foetus at 9 months of pre-natal development. The nerve-endings are of "en plaque" type. 128 X

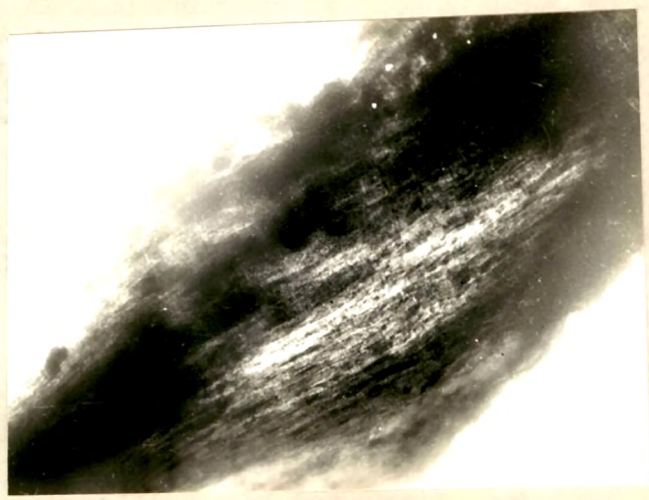

Fig. 10

Longitudinal section showing the histochemical localization of BuChE in the ventral region of the diaphragm of human foetus at 9 months of pre-natal development. The nerve-endings are of "en plaque" type. 128 X

Fig. 11

Longitudinal section showing the histochemical localization of AChE in the dorsal region of the diaphragm of the adult human. The nerve-endings are of "en plaque" type. 128 X

Fig. 12

Longitudinal section showing the histochemical localization of BuChE in the dorsal region of the diaphragm of the adult human. The nerve-endings are of "en plaque" type. 128 X

Fig. 13

Longitudinal section showing the histochemical localization of AChE in the lateral region of the diaphragm of the adult human. The nerve-endings are of "en plaque" type. 128 X

Fig. 14

Longitudinal section showing the histochemical localization of AChE in the ventral region of the diaphragm of the adult human. The nerve-endings are of "en plaque" type. 128 X.