Enzyme histochemistry of skeletal muscle

Part III Neurogenic muscular atrophies

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As discussed in the preceding paper (Dubowitz, 1965), it is possible with histochemical techniques to recognize two fibre types in human skeletal muscle, and to follow the sequence of differentiation of muscle into these fibre types in the course of foetal development. The type I fibres are rich in certain oxidative enzymes such as NADH (nicotinamide-adenine dinucleotide) diaphorase and poor in phosphorylase, while type II fibres are rich in phosphorylase and also other enzymes such as myosin adenosine triphosphatase (ATPase) but poor in the Krebs cycle oxidative enzymes (Fig. 1).

In an application of these methods to pathological muscle (Dubowitz and Pearse, 1961), we observed in two cases of neurogenic atrophy that the isolated or small groups of enlarged or normal-sized fibres seemed to have a uniform enzyme content. It was postulated that these might all conform to one fibre type and might correspond to the Wohlfart 'b' fibres of foetal muscle (Wohlfart, 1937).

Recently Fenichel and Engel (1963) have made a histochemical study of biopsies of the quadriceps from seven cases of infantile spinal muscular atrophy. They measured the fibre diameters of a thousand fibres, staining either light (type I) or dark (type II) for ATPase, and plotted curves for the numbers of fibres against the fibre diameter for each type. They found that there was a decrease in the mean diameter of type I fibres, while type II fibres were either unchanged in size or only slightly reduced. Hypertrophy, when it occurred, was seen almost exclusively in type I fibres. In only one of their patients did some of the enlarged fibres belong to type II.

On the basis of these results they suggested that there was a specific relationship between muscle fibre type and size alterations in infantile spinal muscular atrophy. Type I fibres appeared to undergo severe atrophy and hypertrophy while type II fibres were altered little or not at all. They suggested two possible explanations for this: either (1) that both

types of fibre were denervated but type I fibres were more susceptible to change or (2) that fibre types were related to innervation and that an early selective degeneration of 'type I anterior horn cells' might explain the selective changes in type I fibres.

MATERIAL AND METHODS

Biopsies have been obtained under local anaesthesia from seven children with the severe form of infantile spinal muscular atrophy (Werdnig-Hoffmann's disease) and from eight children with a more benign form of infantile spinal muscular atrophy (Tables I and II). The clinical and histological features of the benign form of infantile muscular atrophy have recently been reviewed (Dubowitz, 1964).

At biopsy two or three separate specimens of each muscle were taken, and rapidly frozen in liquid nitrogen for histochemical study along the lines previously described (Dubowitz and Pearse, 1961, 1964).

In addition to routine histological stains, the following enzymes were studied: NADH diaphorase, NAD-

FIG. 1. Normal muscle. Aged 11. Vastus lateralis. Shows checkerboard pattern of strong and weak fibres. Phosphorylase $\times 100$.

linked lactate dehydrogenase, phosphorylase, and ATPase. Most of the assessments of fibre types were made on serial sections stained for NADH diaphorase and phosphorylase, which were found to give the most consistently reliable results.

Measurements were not undertaken of large numbers of fibres, but the majority of enlarged fibres were measured and also a selection of atrophic ones, in order to obtain a range of respective fibre diameters.

RESULTS

The results are summarized in Tables I and II. With the exception of case 4 all the biopsies showed the classical features of denervation atrophy, with large groups, often whole bundles, of small atrophic fibres and residual isolated or small groups of normal-sized or enlarged fibres. In case 4 the initial biopsy at 10 months showed some bundles which were normal in appearance with normal-sized fibres (25 to 35 μ), and other bundles which were normal in appearance but contained small polygonal fibres (5 to 20 μ) (Fig. 2). A second biopsy at 2 years showed a classical denervation pattern. In case 13 one specimen of muscle was completely normal (triceps 1) while the other two showed unequivocal denervation.

Histochemically there was a variation in pattern, not only from patient to patient, but also from one

specimen to another within the same muscle. The results will be discussed separately for the large fibres and the small fibres. The small proportion of intermediate sized fibres sometimes present are either included with the group to which they approximate in size, or excluded from the assessment.

WERNIG-HOFFMANN'S DISEASE In four of the eight biopsies (cases 1, 3, 6, and 7), one or more specimens showed universally small fibres (Fig. 3). In four biopsies (cases 1, 5, 6, 7) the small fibres showed a mixed population of strongly and weakly reacting fibres with the various enzyme reactions (Fig. 3). These corresponded to type I and type II fibres of normal muscle and seemed to have a similar distribution.

In two biopsies (cases 3 and 4a), the small fibres in all the specimens were predominantly of type I. The second biopsy from case 4 (4b) showed a preponderance of type I fibres in one specimen and a normal distribution in the other two specimens. The biopsy from one patient (case 2) showed a preponderance of type II fibres in one specimen and a normal distribution of type I and type II in the other. In no case did the abnormally large fibres show a consistently mixed population of strongly and weakly reacting fibres comparable to normal muscle.

In three biopsies (cases 3, 4a, and 7) large fibres

TABLE I

INFANTILE SPINAL MUSCULAR ATROPHY (WERNIG-HOFFMANN)

Case	Age	Muscle	Fibre Size (μ)	Large Fibres ¹			Small Fibres				Clinical Course
				NADH+ Phosph- Type I	Phosph+ NADH- Type II	Mixed Type I Type II	NADH+ Phosph+ Size (μ)	NADH+ Phos- Type I	Phosph+ NADH- Type II	Mixed Type I and Type II	
1 A.B.	11 wk.	Quad. 1	50-60	+			10-15			+	Died 4½ mth.
		Quad. 2	—				10-25			+	
2 M.T.	7 mth.	Quad. 1	25-40	+			5-15				Died 9 mth.
		Quad. 2	25-35	+			5-10		+		
3 M.L.	8 mth.	Quad. 1	60-90		+		5-22	+			Died 17 mth.
		Quad. 2	40-60		+		5-15	+			
		Quad. 3	—				5-20	+			
4 K.C.	a 10 mth.	R. Quad. 1	25-35		+		5-20	+			See below*
		R. Quad. 2	25-35		+		5-15	+			
		L. Quad. 1	50-60		+		10-15			+	
	b 2 yr.	L. Quad. 2	30-80				20-30			+	
		L. Quad. 3	40-60		+		10-15	+		+	
5 J.H.	3 mth.	Quad. 1	20-40				10-15			+	Died 4 mth.
		Quad. 2	20-40				10-15			+	
		Quad. 3	20-50		(+)	+	10-15			+	
6 D.P.	11 wk.	Quad. 1	40-50	+			10-20			+	Died 5 mth.
		Quad. 2	40-50	+			10-25			+	
		Quad. 3	—				10-20			+	
7 L.B.	6 mth.	Quad. 1	30-40		+		10-15			+	Died 8 mth.
		Quad. 2	—				10-20			+	
		Quad. 3	—				10-20			+	

¹A '+' refers to the predominant fibre type (more than 80% of fibres).

*Slight deterioration. Still alive, aged 3½ years. Unable to stand.

NADH + Strong reaction for NADH diaphorase.

PHOSPH - Weak reaction for phosphorylase.

TABLE II

BENIGN TYPE OF SPINAL MUSCULAR ATROPHY

Case	Age (yr.)	Muscle	Large Fibres ¹					Small Fibres				
			Fibre Size (μ)	NADH+ Phosph- Type I	Phosph+ NADH- Type II	Mixed Type I and Type II	NADH+ Phosph+ Type I	Fibre Size (μ)	NADH+ Phosph- Type I	Phosph+ NADH- Type II	Mixed Types I and Type II	NADH+ Phosph+ Type II
8 J.T.	12	Gastro.	50-150		+			5-10	Weak	Weak	+	
9 J.J.	17	Quads. 1	—					5-15	Weak			
		Quads. 2	—					5-15				Weak
10 J.A.	12	Gastro.	75-125				W	5-40	(+)		+	
11 J.B.	12	Gastro.	150-190		+			5-20			+	
12 S.G.	15	Quads.	60-80		+			5-15			+	
13 M.K.	8	Triceps 18	50-90			+		—				
		Triceps 2	50-60		+			10-15		(+)	+	
		Triceps 3	50-150		+			10-20			+	
14 D.T.	5	Quads.	25-50				+	5-15				Weak
15 J.W.	6	Quads.	60-150		+			5-20	(+)		+	

¹A '+' refers to the predominant fibre type (more than 80% of fibres).

NADH + Strong reaction for NADH diaphorase

PHOSPH - Weak reaction for phosphorylase

FIG. 2. Quadriceps. Werdnig-Hoffman's disease. Case 4a. Unusual pattern of normal looking bundles of large fibres and small fibres. Haematoxylin and eosin $\times 100$.

FIG. 3. Werdnig-Hoffman's disease. Case 6. Rectus femoris. Universally small fibres comprising strongly and weakly reacting fibres. Phosphorylase $\times 50$.

were predominantly of type II (Fig. 4); in three cases (1, 2, and 6) type I preponderated (Fig. 5).

Of the remaining two patients, one (case 5) showed fibres rich in phosphorylase as well as NADH diaphorase in two specimens and a mixture of type I and type II fibres in the third, with a slight excess of type I fibres. The other patient (case 4b) showed a preponderance of type II fibres in two specimens, while in the third the fibres reacted strongly for both phosphorylase and NADH diaphorase (Figs. 6 and 7).

BENIGN FORM OF INFANTILE MUSCULAR ATROPHY
In five patients (8, 10, 11, 13, 15), the small fibres

comprised a mixed population of type I and type II fibres. In case 12, the phosphorylase reaction was weakly positive and showed a subdivision into two types, but the NADH diaphorase was negative. In cases 9 and 14 the reaction was weak for both enzymes and assessment of any subdivision into fibre types was impossible.

In no less than five of the eight biopsies (cases 8, 11, 12, 13, and 15) the large fibres were predominantly type II (Figs. 8 and 9). In case 9 the muscle was composed of atrophic fibres only. In case 10 the fibres reacted weakly for both phosphorylase and NADH diaphorase. In case 13 there was an interesting variation between the three differ-

FIG. 4. *Werdnig-Hoffman's disease. Case 3. Quadriceps. Large fibres strongly reacting (type II). Phosphorylase $\times 100$.*

FIG. 5. *Werdnig-Hoffman's disease. Case 6. Quadriceps. Large fibres strongly reacting (type I). NADH diaphorase $\times 100$.*

FIG. 6. *Werdnig-Hoffman's disease. Case 4b. Quadriceps. Large fibres uniformly strong. NADH diaphorase $\times 100$.*

FIG. 7. *Werdnig-Hoffman's disease. Case 4b. Serial section to Fig. 6. Large fibres uniformly strong. Phosphorylase $\times 100$.*

ent specimens from the same muscle. One specimen showed normal looking muscle composed of two fibre types as in mature muscle. However, the fibres of smaller diameter (50 to 60 μ) were rich in phosphorylase and poor in NADH-diaphorase (type II), while the larger fibres (70 to 90 μ) were mainly rich in NADH diaphorase and poor in phosphorylase (type I). This correlation with relative fibre size is the opposite to that seen in normal muscle. The other two specimens showed the typical histological pattern of denervation and histochemically the larger fibres were predominantly type II. In one patient (case 14) the large fibres gave a very strong reaction for both NADH diaphorase and phosphorylase.

DISCUSSION

In both Werdnig-Hoffmann's disease as well as the more benign form of infantile spinal muscular atrophy, the small atrophic fibres usually comprise a mixture of strongly and weakly reacting fibres for a particular enzyme reaction, with a similar distribution to normal muscle. This suggests that these very small fibres are the result of atrophy of fully differentiated, mature muscle and not an arrest in the development of embryonic muscle, as has often been postulated in the past.

Moreover, from observations on enzymic differentiation in normal foetal muscle (Dubowitz

FIG. 8. *Benign infantile muscular atrophy. Case 15. Quadriceps. Large fibres strong (type II). Phosphorylase $\times 100$.*

FIG. 9. *Benign infantile muscular atrophy. Case 15. Same muscle as Fig. 8. Large fibres weak (type II). NADH diaphorase $\times 100$.*

1965), the muscle must have reached a gestation of more than 26 weeks in order to have an equal proportion of the two fibre types. One can thus postulate that if a case of Werdnig-Hoffmann's disease is already paralysed at birth, the atrophic process must have started after the 26th week of gestation.

The abnormally large fibres in both clinical forms of muscular atrophy do not conform to normal muscle in enzyme pattern. The majority of biopsies showed a preponderance of fibres rich in phosphorylase and poor in NADH diaphorase, thus corresponding to the type II fibres of normal muscle. This is the exact opposite of the observations of Fenichel and Engel (1963), who found that the abnormally large fibres were almost invariably type I. Moreover, in some biopsies the large fibres showed a uniformly high content of both phosphorylase and NADH diaphorase, suggesting a possibly early foetal pattern of uniform activity for both enzyme systems.

While it was originally suggested (Dubowitz and Pearce, 1961) that these abnormally large fibres might correspond to Wohlfart 'b' fibres, I now think this unlikely for a number of reasons. In the majority of the biopsies the large fibres corresponded to type II, while we have demonstrated in foetal muscle that Wohlfart's 'b' fibres correspond to type I fibres in distribution (Dubowitz, 1965). In some biopsies there were large groups, or even practically whole bundles, of these large fibres. The Wohlfart 'b' fibres always form a small proportion of the total fibres and are scattered singly throughout the muscle and not in clusters.

Smith (1965) has recently studied the histochemical changes following denervation in the rat. The pattern is completely different from that of

diseased human muscle. The reason is probably the acuteness and totality of the denervation in the experimental animal, while in the human the denervation process is a slow and possibly selective one and may in addition be accompanied by reinnervation. Abnormally large fibres are not seen in the experimental animal. It is thus not possible to draw any parallel between the human disease and the experimental animal.

SUMMARY

Enzyme histochemical studies have been done on biopsies from seven patients with severe infantile spinal muscular atrophy (Werdnig-Hoffmann's disease) and eight patients with benign infantile spinal muscular atrophy.

The atrophic fibres usually consist of two fibre types with a similar distribution to normal muscle. This implies that they are atrophied fully mature muscle and not arrested embryonic muscle. Previous observations on foetal muscle suggest that the atrophic process must have started after the 26th week of gestation. In four of the cases of Werdnig-Hoffmann's disease, at least one specimen was composed of universally small fibres only.

The abnormally large fibres usually belong to one enzyme type. Most frequently they corresponded to type II but some were type I and others had a high content of both enzyme systems, suggesting an early foetal pattern of uniform activity for both enzyme systems. Reasons have been given why it is unlikely that these large fibres correspond to Wohlfart 'b' fibres.

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