Histochemically demonstrable fibre abnormalities in normal skeletal muscle and in muscle from carriers of Duchenne muscular dystrophy

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SUMMARY Deltoid muscle was removed at motor point biopsy from 10 female relatives of patients with Duchenne muscular dystrophy and from seven others, with no evidence of neuromuscular disease. Transverse cryostat sections of the muscle from each case were stained for reduced diphosphopyridine nucleotide diaphorase and it was found that all contained varying numbers of degenerating type 1 fibres. The percentage of abnormal fibres in the type 1 fibre population was then calculated for each case and it was found that the muscles from the patients with dystrophic relatives contained considerably higher percentages of abnormal fibres, which also showed more severe degeneration, than did the muscles from the normal cases. There was no absolute correlation between serum creatine kinase levels and degree of pathological change, though patients with most severe changes in their muscles had abnormally high serum creatine kinase levels. It is suggested that histochemical studies could be a useful addition to the present diagnostic tests for the carrier state in Duchenne muscular dystrophy.

The occurrence of focal histological abnormalities in the muscle of carriers of Duchenne muscular dystrophy has been well documented (Dubowitz, 1963a; Emery, 1963, 1965a, b; Stephens and Lewin, 1965; Pearce, Pearce, and Walton, 1966; Radu, Migea, Török, Bordeianu, and Radu, 1968; Ionasescu, Vuia, Luca, and Popa, 1969) such abnormalities also occurring in normal muscle (Adams, 1968). Histochemical investigations have also revealed occasional abnormal fibres in the muscle of some carriers (Engel and Cunningham, 1970; Roy and Dubowitz, 1970) and it is likely that such abnormal fibres also occur in normal muscle. A histochemical comparison of normal and carrier muscle fibres may, therefore, be of use in deciding the significance and possible diagnostic importance of the observed abnormalities.

METHODS

Motor point biopsies (Coërs and Woolf, 1959) were performed under local (cases 1-11, 16, and 17) or general anaesthetic (cases 12-15) on the deltoid muscle of 11 females with a family history of Duchenne muscular

dystrophy. Of these six were possible carriers, two were probable carriers, and three definite carriers (Pearce Pennington, and Walton, 1964; Thompson, Murphy, and McAlpine, 1967). Deltoid muscle was removed in the same way from six volunteer male patients, four undergoing orthopaedic operations (cases 12-15) and two husbands of dystrophic carriers (cases 16 and 17) (Table).

Several small pieces of muscle cut at random from each biopsy were rapidly frozen in liquid nitrogen and subsequently stored in small polyethylene containers in the deep freeze. Transverse sections of the muscle were cut at 6 μ m on a cryostat with a cabinet temperature of -20°C and were attached directly to alcohol cleaned microscope slides. The sections were allowed to dry briefly at room temperature and were then stained for reduced diphosphopyridine nucleotide diaphorase (DPNH-D) (Scarpelli, Hess, and Pearse, 1958). The sections were fixed in 4% formaldehyde in normal saline overnight, treated with acetone to remove monoformazan, washed in distilled water, and mounted in polyvinylpyrrolidone (Burstone, 1957). The percentage of abnormal type 1 fibres was calculated from a random examination of 300 type 1 fibres from each case.

Creatine kinase levels in venous blood taken at rest were estimated by the method of Hughes (Hughes, 1962) modified for the autoanalyser (Siegel and Cohen, 1966).

TABLE

SUMMARY OF RESULTS FROM MUSCLE ENZYME HISTOCHEMISTRY AND CREATINE KINASE ESTIMATIONS

Case no.	Age (yr)	Genetic carrier status	Creatine phosphokinase (i.u./l.*)	% Abnormal type 1 fibres
1	31	Possible	123.0	8.2
2	46	Definite	259.0	6.5
3	37	Definite	390.0	9.1
4	24	Possible	26.1	15.7
5	47	Definite	97.7	6.7
6	32	Probable	35.1	4.1
7	22	Possible	32.3	13.5
8	36	Possible	250.0	2.7
9	18	Possible	38.2	0.7
10	38	Probable	24.6	4.7
11	33	Possible	—	5.4
12	42	Normal	—	0.7
13	21	Normal	—	0.4
14	12	Normal	32.9	0.0
15	15	Normal	—	0.2
16	34	Normal	—	1.2
17	36	Normal	—	0.9

*Normal values: females <60 i.u./l., males <80 i.u./l.

RESULTS

The muscle from all the cases studied showed well defined normal differentiation into types 1, 2, and intermediate fibres, the three being present in a normal random mosaic. In all cases, the great majority of the type 2 and intermediate fibres were of normal size and structure, though a few showed slight intermyofibrillar network disruption of doubtful significance. The type 1 fibres were affected to different degrees in the various cases, there being no abnormal fibres in case 14 but 15.7% in case 4 (Fig. 1). Cases 1-8, 10, and 11 had higher percentages of abnormal fibres (2.7 to 15.7%) than did the other seven cases (0 to 1.2%). The structure of the abnormal type 1 fibres varied between two extremes:

1. Somewhat atrophic angular fibres with coarse and fragmented intermyofibrillar networks and well marked subsarcolemmal enzyme activity around the whole fibre periphery (Figs. 2, 3, 4).

FIG. 1. Transverse section of deltoid muscle from case 4. Abnormal type 1 fibres (arrowed) are present within a normal mosaic of type 1 (dark) and type 2 (light) fibres. Reduced diphosphopyridine nucleotide diaphorase (DPNH-D), $\times 500$.

FIG. 2

FIG. 3

FIGS. 2-4. Transverse sections of deltoid muscles from cases 1, 3, and 5. The small angular type 1 fibres have heavy subsarcolemmal enzyme activity (arrowed in Fig. 2) and coarse intermyofibrillar networks. DPNH-D, $\times 1,000$.

2. Fibres of mean diameter or above with the great proportion of the intermyofibrillar network of normal thickness. The network was, however, grossly disturbed, large areas being completely devoid of enzyme activity. There were a few subsarcolemmal enzyme masses and also aggregations of intense activity scattered throughout the fibre (Figs. 5, 6, 7).

All the definite and probable carriers were found

FIG. 4

to have more abnormal fibres than the normal group (cases 12-17). The possible carriers all had percentages of abnormal fibres above those of normals with the exception of case 9, which was well within the normal range.

DISCUSSION

The abnormalities observed in both the normal and carrier muscle fibres were very similar, although the more severe atrophic changes were seen only in the carrier muscle. The abnormally heavy deposits of oxidative enzyme activity probably represent an increase in number, or abnormality of the mitochondria, since reduced diphosphopyridine nucleotide diaphorase is known to be located in them (Novikoff, Shin, and Drucker, 1961). This view is supported by recent electron microscopical studies of carrier muscle in which there were focal aggregations of abnormally large numbers of mitochondria, some showing increase in size (Milhorat, Shafiq, and Goldstone, 1966; Roy and Dubowitz, 1970). In one of these studies (Roy and Dubowitz, 1970) the histochemical stains in one case showed similar changes to those found by us, some of the type 1 fibres having irregularly distributed masses of oxidative enzyme activity attributed to the presence of mitochondrial aggregations. The electron microscopical studies have also revealed areas of focal degeneration with loss of myofibrils and destruction of the intermyofibrillar network seen in all the histochemically abnormal fibres. There seems little doubt that the observed histochemical changes in the muscle fibres are the result of a degenerative process, active in both normal and carrier muscle but more extensive and severe in the latter. The spectrum of

FIG. 5

FIG. 7

FIG. 6

FIGS. 5-7. Transverse sections of deltoid muscles from cases 7, 8, and 10. The type 1 fibres have only occasional subsarcolemmal enzyme masses and there are areas devoid of enzyme activity. DPNH-D, $\times 1,000$.

changes noted in the abnormal fibres in this study and in previous electron microscopical studies suggest that the degenerating fibre first increases in size with splitting and separation of myofibrils associated with disruption of the intermyofibrillar network and subsequently atrophies with severe loss of myofibrils and clumping of the intermyofibrillar network. The fact that the histochemical changes are mostly confined to the type 1 fibres is very difficult to explain, though it has been suggested

that selective involvement of fibre types in some myopathies is due to the metabolic and structural difference between the two fibre types as demonstrated histochemically (Engel, 1965, 1970). Although the muscle from the definite carriers showed the most severe atrophic changes in type 1 fibres, they did not have the greatest percentage of abnormal fibres, these being found in the younger females in the group of possible carriers. The presence of large numbers of abnormal fibres in muscle from possible carriers was also noted in a previous study (Roy and Dubowitz, 1970). The cases with the highest numbers of affected fibres (cases 4 and 7) had the lowest levels of creatine phosphokinase (CPK), whereas case 8, with a small percentage of abnormal fibres, had a high CPK. The cases with the most severe changes (cases 2 and 3) did, however, have the highest CPK values, but case 8 with a high CPK, had only slight fibre abnormalities. Similar inconsistencies were found in previous studies (Dubowitz, 1963b; Roy and Dubowitz, 1970).

The percentage of abnormal type 1 fibres in case 16 was higher than that of the other normal muscles, a finding which could be explained by the presence of an abnormally large number of collateral sprouts of the terminal nerve fibres in methylene blue preparations (Evans, Haynes, Morris, and Woolf, 1971) from this case. The possibility exists that there is a difference in abnormal type 1 fibre percentages in normal muscle from different sexes, a fact which we have not been able to investigate owing to a lack of normal female muscle. It seems unlikely, however, that this would explain the large differences seen between the male controls and the dystrophic carriers, particularly as one of these

(case 9) has a percentage of abnormal fibres well within the control range.

On the basis of this study, with a rather limited amount of normal material, all the carriers and potential carriers, with the exception of case 9, could be classed as having abnormally large numbers of degenerating muscle fibres. Two cases (cases 4 and 7) would appear to be unequivocally abnormal, even though other conventional tests for the carrier state were completely normal. Histochemistry could, therefore, be a useful adjunct to the existing tests for the carrier state in Duchenne muscular dystrophy, having the advantage of detecting the more subtle abnormalities in muscle fibres not readily demonstrated by conventional histological techniques.

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