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Muscle nuclear changes in fetuses at risk for Duchenne muscular dystrophy

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SUMMARY Muscle nuclear size was found to be significantly greater in fetuses at risk for Duchenne muscular dystrophy than in normal male fetuses of comparable gestational age. This supports the contention that the disease is already manifest in utero by the second trimester of pregnancy.

The muscle lesions in X-linked Duchenne muscular dystrophy (DMD), including preclinical cases, are well documented (Pearson, 1962; Hudgson *et al.*, 1967; Bradley *et al.*, 1972). Toop and Emery (1974) have also reported changes in muscle histology in fetuses at risk for DMD, and Webb (1974) suggested that muscle cell death in early fetal life could explain the pathogenesis of this disease.

Recently Vassilopoulos *et al.* (1976) reported a significant increase in muscle nuclear size in cases of DMD. This was explained as being probably a reflection of changes in nucleocytoplasmic relations. The present study was undertaken to see whether similar changes are also present in fetal dystrophic muscle.

Materials and methods

Muscle tissue from eight male fetuses at risk for DMD was examined. In four cases (Nos. 979, 1018, 75/479, and 76/60) the mothers were definite carriers and in the remaining four the mothers were possible carriers but at high risk (greater than 1 in 10) of having an affected son. In each case the sex of the fetus was established before abortion by sex chromatin and fluorescent studies on uncultured amniotic fluid cells and from karyotype analysis of cultured amniotic fluid cells. The results were compared with the findings in eight male fetuses of comparable gestational age obtained at abortion performed for social reasons and where there was no history of any neuromuscular disorder.

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The gestational age of the fetuses was estimated by crown-rump (Hamilton and Boyd, 1962) and heel-toe (Streeter, 1921) measurements. Frozen sections 10-μ thick stained with haematoxylin and eosin were examined (Figure). The cross-sectional areas of at least 100 nuclei closely apposed to the surface of transversely sectioned muscle fibres were estimated by planimetry at a final magnification of $\times$ 100. The nuclei were selected for measurement in this way to

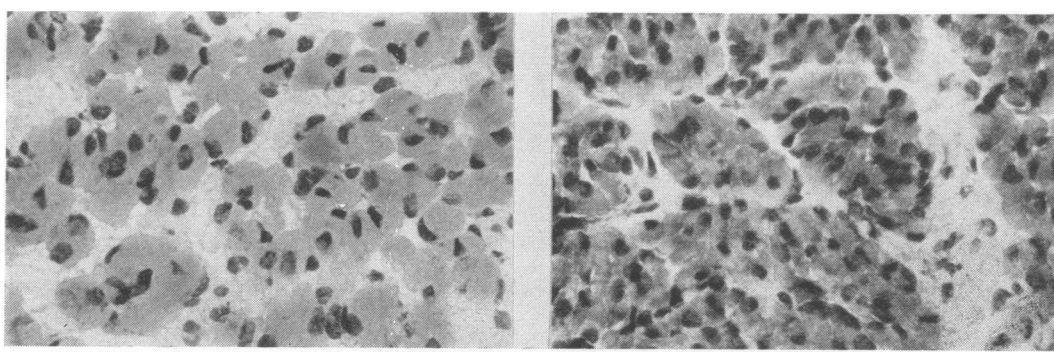

Fig. *Transverse sections of muscle of fetus at risk for Duchenne muscular dystrophy (left) and of normal fetus of same gestational age (right). Cryostat sections. (Haematoxylin and eosin $\times$ 500.)*

Table *Mean (±SD) muscle nuclear size in normal fetuses (controls) and in fetuses at risk for DMD*

Case No.	*Mother's status*	*Gestational age (weeks)*	*Nuclear size (μm^2)*
		Controls	
837	N	16	32·0 ± 8·3
73/125	N	21	34·1 ± 8·4
73/101	N	22	29·9 ± 7·2
73/130	N	18	27·2 ± 5·6
2105	N	19	29.2 ± 6·4
73/127	N	22	27·8 ± 5·6
909	N	16	24·9 ± 4·8
72/22	N	22	26·8 ± 4·9
		Fetuses at risk	
979	D	20	33·2 ± 8·4
1018	D	21	34·1 ± 7·8
75/479	D	18	28·1 ± 7·3
76/60	D	22	39·8 ± 8·4
684	P	16	31·1 ± 8·8
734	P	16	40·8 ± 9·3
73/375	P	19	35·9 ± 7·7
599	P	21	37·1 ± 11·6

N = normal. D = definite carrier. P = possible carrier.

eliminate fibroblast, pericyte, endothelial cell, and satellite cell nuclei. Nuclear volume was not estimated since this requires certain assumptions to be made about the irregular shape of the muscle nucleus (Franke and Schinko, 1969). All measurements were made 'blind'—that is, without any knowledge of the source of the material.

Results

The results of the study are shown in the Table. Though there is overlap in the individual results obtained the overall mean size of nuclei in the group at risk for DMD (35·0 $\mu m^2 \pm 4·2$) is greater than in the matched controls (28·9 $\mu m^2 \pm 2·9$), the difference being statistically significant ($P < 0·002$). A proportion of the fetuses at risk would be expected to be normal, but the results showed no evidence of bimodality and the lower values of fetuses at risk fell into the upper half of the control values. However, considering the high variability of the muscle fibre nuclear size it is difficult to draw any definite conclusions about this.

Discussion

Muscle weakness in Duchenne muscular dystrophy first becomes evident at about the age of 3 to 5 years. Pearson (1962), however, examined muscle tissue from a 2-month-old boy with preclinical Duchenne muscular dystrophy and found widespread hyalinization of the muscle fibres and an increased variation in their size. Bradley *et al* (1972), in a biopsy of muscle from a 17-day-old boy who later developed DMD, confirmed Pearson's findings and pointed out that pathological changes might well be evident even earlier. In a study of muscle histology in fetuses at risk for DMD, Toop and Emery (1974) in fact reported abnormalities similar to those described in preclinical cases and they concluded that certain histological changes were already evident in utero. In the present study the enlargement of muscle nuclei seen in fetuses at risk for DMD and the increase in muscle nuclear size in patients with DMD (Vassilopoulos *et al.*, 1976) probably reflect the same underlying pathogenic process.

Nuclear changes in a variety of tissues have been reported in a number of diseases and experimental conditions but their significance is still controversial (Heiberg, 1957; Guimarães, 1971). The size of muscle fibre nuclei has been variously related to cellular hypertrophy (Doljanski, 1960; Goss, 1964), altered ionic environment (Davies and Spencer, 1962), and the degree of muscle contraction (Franke and Schinko, 1969). Enlargement of the nucleus seems to be one of the earliest and most consistent responses to alterations in nuclear environment.

Much information about nucleocytoplasmic relations has been gained from nuclear transplantation experiments in amphibia (Gurdon, 1968, 1970), and changes in nuclear size may well be related to alterations in gene expression (Lewin, 1974). This may explain the changes in muscle nuclear size in DMD and in fetuses at risk for DMD. In any event the findings reported in this study provide further support for Toop and Emery's (1974) suggestion that this disorder is already manifest in utero by the second trimester of pregnancy.

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