ficiale. If we compare AChE localization in the optic tectum of reptiles with that of amphibians and birds[7,10,19–21], a clear evolutive sequence is observable. In all cases, the enzymatic activity is prevailingly localized in the stratum fibrosum and griseum superficiale, but its distribution pattern shows progressive lamination in connection with the concurrent development and differentiation of the same stratum, which reaches a maximum in the birds[22,23]. This process of lamination is emphasized by the AChE distribution pattern in the series frogs-lizards and turtles-alligator-birds, while the snakes seem to possess a distribution pattern not clearly related to the structural organization of the stratum fibrosum and griseum superficiale. In this connection, however, one must remember that the optic tectum of snakes, and in particular the stratum fibrosum and griseum superficiale, shows reduction in differentiation and structural organization in comparison with the other reptiles[2,18,24]. Since the stratum fibrosum and griseum superficiale constitutes the main receptive layer for sensitive discharge, mainly the retinal one, the features of AChE distribution strongly suggest a precise correlation between the enzymatic activity and the mechanisms of reception and modulation of sensitive input to the tectum. The uneven BuChE distribution in reptilian optic tectum confirms the extreme species-dependence of this enzymatic activity, as previously pointed out in birds and mammals[25].

The preferential MAO localization at level of fibrous layers of the tectum seems to be a common feature in vertebrate optic tectum; a similar kind of MAO distribution exists in teleosts[26], amphibians[7] and birds[10,21]. In addition, the distribution pattern observed in reptilian optic tectum is in good agreement with the localization of serotonine and catecholamine-containing nerve terminals, as revealed by the method of formaldehyde-induced fluorescence[27–29].

The dorsal tectal areas and in particular the stratum fibrosum and griseum superficiale are provided with high activity of oxidative enzymes as previously pointed out for SDH in other reptilian species[9,30]. In reptilian optic tectum, a connection seems to exist between these enzymes and AChE distribution; in fact the areas provided with high AChE activity show substantial activity of LDH and/or SDH. This kind of relationship is usually present in many nervous regions of other vertebrates[25]. In conclusion, the optic tectum in reptiles, as in other vertebrates[6,7,9,10,19–21], exhibits a close relationship between the level of structural and functional organization, and the distribution patterns of some enzymes connected with energy metabolism and other enzymes connected with the specific neural function.

The results of the present work, together with other studies on enzyme distribution in the vertebrate nervous system, provide further support for the research-line of the chemical mapping of the brain, as stated by Friede[25].

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Nuclear changes in cultured human dystrophic muscle

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Summary. Nuclear size in myotubes in cultured human dystrophic muscle has been found to be significantly greater than in normal muscle. These findings are discussed in relation to the pathogenesis of muscular dystrophy.

Significant enlargement of the muscle nucleus has been observed in biopsies from children with Duchenne muscular dystrophy (DMD)[3] and in some male fetuses at risk for DMD[4]. This finding was interpreted in terms of modified nucleo-cytoplasmic relationships. The present study was undertaken to determine if similar changes in the muscle fibre nucleus occur during the early stages of dystrophic muscle development in vitro.

Materials and methods. Myogenic cell lines were prepared from fresh muscle biopsies of 6 normal and 2 dystrophic children. The samples were processed as soon as possible after biopsy and were first washed in phosphate buffered saline solution containing 100 units/ml Penicillin, 200 mg/ml Streptomycin and 2.5 µg/ml Amphotericin B (Mg and Ca ion free). The explants were clotted using a 1:1 mixture of filtered chick embryo extract and cock plasma for a few minutes. Once clotting had taken place, the explants were overlaid with Hams F-10 containing 10% fetal calf serum (FCS) and the same concentration of antibiotics. Explants with well developed myotubes were used for secondary cultures. These were set up following trypsinisation (0.25% trypsin in Dulbecco – pH 7.4). The differentiation of myoblasts was examined after 15 and 25 days culture in 10% FCS on coverslips subsequently stained with haematoxylin and eosin.

1 The authors wish to thank Mrs L. McGregor and Miss L. Andruzczenko for technical assistance. This work was supported by the Muscular Dystrophy Group of Great Britain. D. V. is in receipt of a scholarship from the State Scholarship Foundation of Greece.
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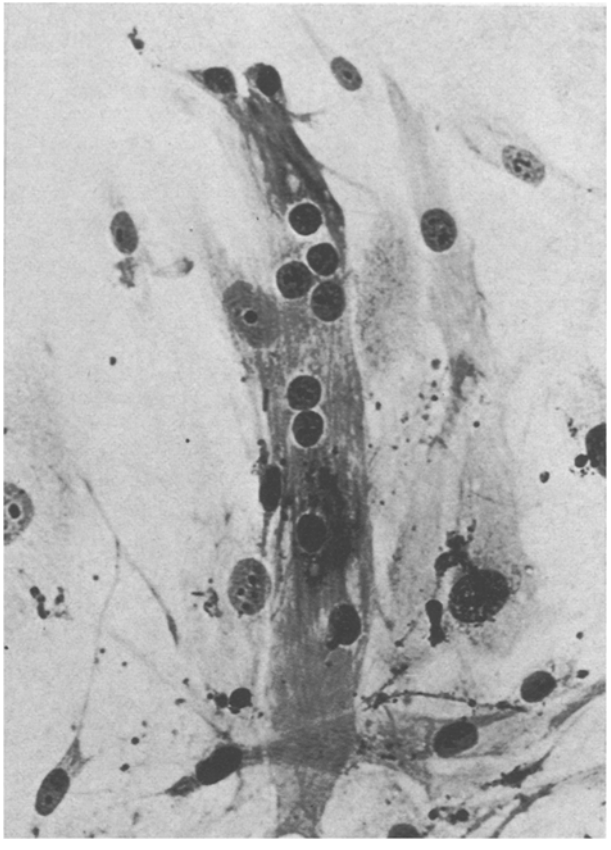

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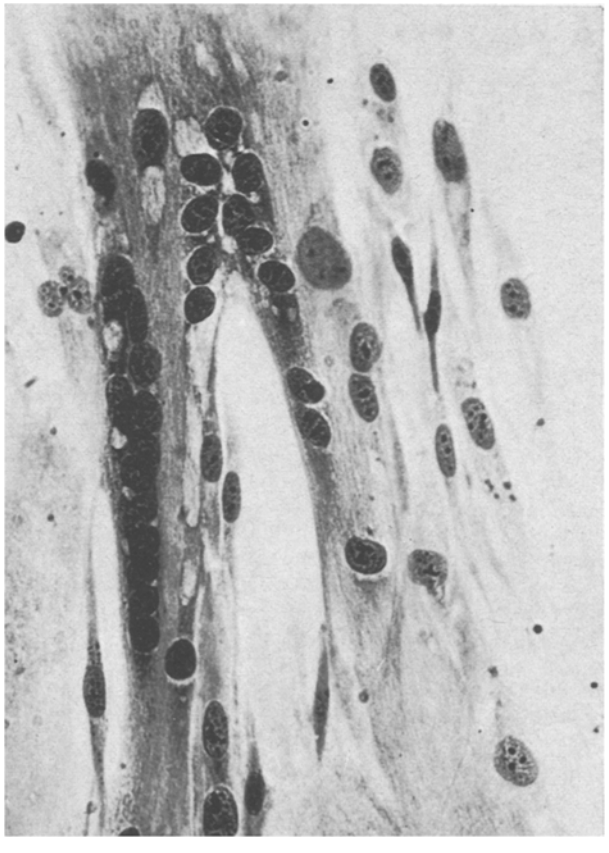

b

Myotubes from a normal *A* and dystrophic *B* muscle after 15 days in subculture. Haematoxylin and eosin. ×450.

Nuclear size in myotubes from normal and dystrophic muscle cultured in vitro

	Identification	Nuclear size (in μm^2) 15 days	25 days
Controls	S. D.	21.8 ± 3.1	19.9 ± 2.6
	N. J.	22.4 ± 2.9	23.0 ± 2.9
	C. R.	23.8 ± 3.0	20.3 ± 2.9
	W. D.	21.9 ± 2.6	20.8 ± 2.7
	W. C.	21.2 ± 2.3	22.5 ± 3.0
	A. M.	20.9 ± 1.9	20.5 ± 2.2
Dystrophic	A. G.	25.7 ± 3.2	23.3 ± 2.9
	A. W.	24.1 ± 3.0	24.3 ± 2.8

The results are expressed as mean area ± SD.

All slides were photographed so that the nuclear area, in a random selection of 15 myotubes from each preparation[5], could be measured by planimetry (final magnification × 1000). All measurements were made 'blind' without knowledge as to the source of the material. No measurements were made of monoculeated myoblasts since these are essentially indistinguishable from fibroblasts. Student's t-test was used for the statistical evaluation of the results.

Results. The results are summarized in the table. At 15 days the mean nuclear size in the dystrophic myotubes (24.9 ± 1.1 μm^2) is significantly greater ($p < 0.02$) than that of the normal myotubes (22.0 ± 1.0 μm^2). At 25 days the difference between dystrophic (23.8 ± 0.7 μm^2) and normal (21 ± 1.2 μm^2) muscle nuclei is also statistically significant ($p < 0.05$) (figure).

Discussion. Skeletal muscle from patients with muscular dystrophy exhibits striking histological changes. On the contrary dystrophic muscle in tissue culture exhibits little if any morphological difference from normal[6–9]. The exact role of the nucleus in muscle fibre function is not yet fully understood. The genetic nature of DMD suggests some nuclear involvement, but Peterson[10] reported that the nucleus, at least in mouse dystrophy, may not play an important role in pathogenesis. Using a technique of chimaera formation, he found that although some fibres had only dystrophic nuclei there was little or no degeneration of the fibres whereas muscle containing no dystrophic nuclei sometimes showed pathological changes. It has been reported[5] that the number of nuclei in dystrophic myoblasts is not significantly different from normal muscle. Our results indicate that the nuclear size in dystrophic myotubes is significantly greater than in normal tissue cultured under identical conditions. This morphological difference between normal and diseased muscle would not be detectable by qualitative techniques as used by Bishop at al.[5]. The observed enlargement seems to reflect the same underlying pathogenic process as is observed in muscle biopsies from patients with DMD and in some fetuses at risk for DMD and it probably reflects modifications in nucleo-cytoplasmic interactions. In any case, our results suggest that the observed enlargement of muscle nuclei found in dystrophic muscle in vitro is present even in the early stages of muscle fibre differentiation.

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