MYOSIN ATPase ACTIVITY IN MULTIFIDUS MUSCLE FROM CASES OF LUMBAR SPINAL DERANGEMENT

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Biopsies of lumbar multifidus muscles were obtained at operation on seventeen patients aged from fifteen to fifty-eight with lumbar spinal derangement, and further material was taken from the cadavers of three subjects aged from nineteen to fifty-one. Sections were prepared to show the presence of ATPase activity, so distinguishing Fast from Slow types of muscle fibre. The normal mosaic pattern arising from the intermingling of fibres from Fast and Slow motor units was seen in sections from cadaveric material and from many of the biopsies. With age and limited lumbar flexibility, the Fast fibres became relatively smaller but with increasing variation in size, suggesting a reduced capacity for phasic activity. The presence of positive root signs was associated with a greater proportion of Slow fibres, and in some patients with the occurrence of atrophied Fast fibres, giving rise to differences in the populations of the two fibres in neighbouring fascicles. The results suggest that multifidus adopts an increasingly postural role with advancing age and with disabling lesions of the lumbar spine.

In cases of lumbar spinal disorder dysfunction of muscle is common, but it is often far from clear whether the change in mechanical function of the spine is due to pain, denervation of the spinal muscles or ligamentous laxity, or whether it is secondary to a mechanical derangement of the vertebral joints (Hult 1954, Lettin 1967, Horal 1969, Newman 1973). Few studies of lumbar spinal muscles have been made. This investigation was carried out to assess changes in multifidus from patients undergoing operations for intervertebral disc prolapse, spondylolisthesis, lumbar instability or lumbo-sacral degenerative change with root involvement.

Multifidus was chosen for study because it is the component of erector spinae which mainly controls movements between individual segments (Morris, Benner and Lucas 1962; Pauly 1966; Jonsson 1970; Donisch and Basmajian 1972; Waters and Morris 1972). Like the muscles of the limbs, it contains populations of both fast-twitch and slow-twitch fibres (Eberstein and Goodgold 1968). These two main types of muscle fibre can be distinguished not only by their mechanical responses to stimuli (Eberstein and Goodgold 1968, McComas and Thomas 1968, Buchthal and Schmalbruch 1970, Warmolts and Engel 1972), but also histochemically (Dubowitz 1965; McComas and Thomas 1968; Brooke, Williamson and Kaiser 1971; Burke, Levine, Zajac, Tsairis and Engel 1971; Close 1972). The fibres of an individual motor unit are histochemically uniform (Kugelberg, Edström and Abbuzzese 1970; Brooke *et al.* 1971). Those of a fast-twitch motor unit are supplied by rapidly discharging but readily fatiguable motoneurons, whereas the moto-

neurons supplying slow-twitch units discharge tonically at a lower frequency but fatigue less readily (Eccles, Eccles and Lundberg 1958; Edström and Kugelberg 1968; Grimby and Hannerz 1968; Kugelberg and Edström 1968; Burke *et al.* 1971; Person and Kudina 1972).

Fast fibres rely metabolically on the anaerobic process of glycolysis with the breakdown of adenosine triphosphate, and so are rich in ATPase. Slow fibres metabolise aerobically, are rich in oxidative capacity and show little ATPase activity. There is a third group of fibres, intermediate metabolically (that is, with both glycolytic and oxidative enzymes) but with fast-twitch characteristics (Davies 1972, Muir 1974). From a functional point of view it is the distinction between fast-twitch and slow-twitch fibres which is the main interest, and they can be distinguished on the basis of their ATPase activity (Padykula and Herman 1955, Dubowitz and Pearse 1961). Thus, in multifidus, the presence of slow-twitch fibres, which are primarily tonic in activity, is consistent with its postural role; and that of fast-twitch fibres, which are active during rapid recruitment for short periods, suggests that multifidus can adapt quickly to interactions between the trunk and limbs, preserving the intersegmental stability of the spine on sudden movement and loading.

The distinction between fibre-types is also valuable neuropathologically. Denervation by cutting a peripheral nerve leads to diminution of the size of fibres, primarily affecting fast fibres (Karpati and Engel 1968*a* and *b*, Engel 1970, Kugelberg *et al.* 1970). Soon after denervation there is a process of dedifferentiation: the contraction

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times of fast muscles become prolonged while slow muscles tend to contract more rapidly (Syrový, Gutmann and Melichna 1971; Gutmann, Melichna and Syrový 1972; Lewis 1972). Atrophy of fast fibres is seen in upper motor neurone lesions (Edström 1968), and hypertrophy of slow fibres appears to be related to the degree of spasticity (Edström, Grimby and Hannerz 1973). Disuse leads to atrophy, generally of both types but according to some reports, mainly of slow fibres (Karpati and Engel 1968a; Patel, Razzak and Dastur 1969). However, in the quadriceps femoris of cases of long-standing injury of the cruciate ligaments, Edström (1970) found selective atrophy of slow fibres due, he thought, to loss of normal proprioceptive function.

No histochemical studies of pathological change appear to have been made of the erector spinae muscle, although Sulemana and Suchenwirth (1972), Johnson, Polgar, Weightman and Appleton (1973) and Polgar, Johnson, Weightman and Appleton (1973) have studied the distribution and size of the two fibre types in erector spinae from cadavers. No histochemical studies of normal or pathological specimens of multifidus itself have been reported. Accordingly, this work was undertaken to investigate ATPase activity in multifidus from patients with lumbar spinal disorders. A further study was made of patients with scoliosis (Fidler, Jowett and Troup 1974).

MATERIAL

Biopsies of multifidus were taken at operation from seventeen patients with derangement of the lumbar spine and at necropsy from three cadavers within twenty-four hours of death. The age, sex and causes of death of the three cadavers are given in Table I.

Details of sex, age, site and duration of pain, the presence or absence of positive root signs and the diagnosis in the seventeen patients with lumbar spinal derangement were recorded in tabular form.¹ In fourteen out of seventeen patients the presence or absence of the "extension catch" (a painful pause in recovery from the flexed stooping position as the lumbar spine begins to extend) was recorded, and measurements were made of the lumbar posture in maximally flexed and extended positions to assess sagittal mobility, using a goniometer to record the angle subtended by skin contours at the T.12 and S.2 levels.

METHOD

Biopsies of multifidus were made after reflecting the lumbo-dorsal fascia and overlying sacrospinalis from the midline. The tendon of insertion was freed from the spinous process and the muscle dissected from its origin. The muscle was suspended by its tendon, cut transversely and frozen in "isopentane" cooled to -180 degrees Celsius in liquid nitrogen. Transverse sections of $8\ \mu$ thickness were cut in a cryostat at -20 degrees and mounted on cover slips.

The sections were incubated in adenosine 5-triphosphate (Sigma London Chemical Co. Ltd.) in a 0.15 per cent solution buffered in sodium barbitone at pH 9.4 for 15 minutes and then stained, using a method based on that of Gomori (1941). The sections were then photographed on 35-millimetre film (Ilford Microneg. Pan or Ektachrome duplicating film), prints being made on Ilfoprint (DR45L) paper for analysis.

Identification of fibres—Fibres with a high level of myosin-ATPase activity stain darkly compared with those of low activity. Control staining without the addition of ATP to the incubation medium failed to stain any fibres and thus confirmed that the staining technique revealed differences due only to high or low content of ATPase. The two types of fibre were readily identifiable.

TABLE I
DETAILS OF THE FINDINGS IN CADAVERIC MATERIAL

Subject	Sex	Age (years)	Cause of death	Level of specimen	Total fibre count			Cross-sectional areas						Low/High mean-area-ratio
					Low ATPase	High ATPase	Percentage of Low fibres	Low ATPase Number	Low ATPase Mean	Low ATPase C.V.	High ATPase Number	High ATPase Mean	High ATPase C.V.	
C.1	F	19	Road traffic accident	L.5	435	318	58	45	3,360	24%	30	4,890	18%	0.69
				L.5	218	99	69	60	2,990	27%	26	4,550	17%	0.66
C.2	M	32	Road traffic accident	T.6, left	697	210	77	79	1,880	24%	14	1,890	17%	0.99
				T.6, right	649	194	77	62	2,570	24%	25	2,950	32%	0.87
				L.5, left	271	127	68	30	4,680	24%	21	4,200	25%	1.11
				L.5, right	275	188	59	34	4,140	20%	37	4,160	18%	1.00
C.3	M	51	Coronary atherosclerosis	L.5*	188	251	43	14	5,540	38%	38	4,740	36%	1.17
								29	4,670	42%	34	3,150	37%	1.48
				L.5	246	109	69	20	6,070	31%	35	4,560	46%	1.33

The total number of fibres with High and Low ATPase activity counted in each section are shown, with the percentage of Low ATPase fibres: the number of fibres whose areas were measured, the mean area (μ^2) and the coefficient of variation (C.V.) are listed, together with the ratio of the mean areas of Low/High fibres.

* Sacrospinalis.

¹Because of its size this table is not included here but it may be obtained on request.

Counting—Photographic prints of the whole section of muscle at a magnification of $\times 70$ allowed all the fibres to be counted. In the results, the total number of Low and High ATPase fibres are presented, together with the proportion of Low ATPase as a percentage of the total.

Cross-sectional areas—Photographic prints with a magnification of $\times 280$ were made. The two types of fibre were identified and then cut out and individually weighed to the nearest 0.001 gram, equivalent to a true area of $89 \mu^2$, the consistency of the mass of given areas of printing paper being better than 2 per cent. The results are presented as the means (to the nearest $10 \mu^2$), the coefficients of variation ($\frac{\text{standard deviation} \times 100}{\text{mean}}$) for Low and High ATPase fibres in individual sections of muscle and the ratios of the mean areas of the two types of fibre. The distributions of fibre sizes were then plotted in the form of histograms. Fibre sizes were occasionally estimated in more than one area of an individual section.

Accuracy of results

No difficulty was experienced in distinguishing Low and High ATPase fibres for counting purposes. In the higher magnification for cutting out and weighing, any problems in identification were overcome by marking individual fibres before cutting.

Two factors may have affected the estimates of fibre sizes. There were occasionally delays, though never exceeding four hours, between taking and freezing a specimen before section, and shrinkage may have occurred. The second factor was the obliquity of the cross-section. When all fibres appeared oblique the section was discarded, but most contained a small proportion among a majority of clearly transverse fibres. This appeared to be a normal structural feature and such fibres were not excluded. As both factors affected each type of fibre, the errors in comparing the mean areas of the two types were assumed to cancel each other and have been ignored.

RESULTS

The mean values for the size and proportion of the two fibre-types in lumbar multifidus, pooled from the seventeen patients with lumbar spinal derangement and from the three cadaveric subjects, are shown in Table II.

fibres varied as a proportion of the total from 58 to 69 per cent. The mean cross-sectional areas of fibres ranged from $1,880 \mu^2$ to $6,070 \mu^2$. The High ATPase fibres were proportionately larger in the youngest subject (C.1) and smaller in the oldest (C.3), the $\frac{\text{Low}}{\text{High}}$ mean-area-ratio varying from 0.66 to 1.33.

Biopsy specimens from patients with lumbar spinal derangement—The typical mosaic distribution of High and Low ATPase fibres was seen in many of the sections (see Fig. 2). This pattern was lost when the proportion of Low ATPase fibres in the section became large enough for them to appear to be grouped because of the scarcity of High ATPase fibres (see Fig. 3). In such sections, there were major differences between the populations of neighbouring fascicles, some containing no High ATPase fibres at all (L.2, L.3, L.10, L.11, L.12, L.17); but in no instances were High ATPase fibres grouped together.

Low ATPase fibres appeared to be of normal size and outline, their mean areas varying from $1,650 \mu^2$ to $7,980 \mu^2$. High ATPase fibres of up to $7,740 \mu^2$ in mean area were seen but in several sections their size appeared to be smaller than normal—in one section (L.11) a mean of $1,150 \mu^2$ —and their polygonal outlines were in some cases markedly angular (see Fig. 4).

In order to relate the results to the clinical findings, the size and proportion of the two fibre-types were analysed according to age, to the patient's ability to flex the lumbar spine, and to the presence or absence of root signs and of an "extension catch".

Age

For this analysis, the results from the cadaveric material were added to those from biopsies from the patients. The twenty subjects were divided into two groups, a younger group of nine subjects aged fifteen to thirty-two and an

TABLE II

THE MEANS (AND STANDARD DEVIATIONS) OF THE PROPORTION OF LOW ATPASE FIBRES AS A PERCENTAGE OF THE WHOLE, THE MEAN CROSS-SECTIONAL AREAS AND THEIR COEFFICIENTS OF VARIATION (C.V.) OF THE TWO FIBRE TYPES, AND THE RATIO OF THEIR MEAN AREAS, FOR THE BIOPSIES FROM THE SEVENTEEN PATIENTS WITH LUMBAR SPINAL DERANGEMENT AND THE SPECIMENS FROM THE THREE CADAVERIC SUBJECTS

	Ages (years)	Percentage of Low ATPase fibres	Low ATPase fibres		High ATPase fibres		Low/High- mean-area- ratios
			Mean areas (μ^2)	C.V.s*	Mean areas (μ^2)	C.V.s*	
mean	34.80	66.60	4,235	29.35	2,787	34.70	1.79
s.d.	13.03	12.98	1,004	8.42	1,131	11.88	0.72

* $n=20$.

Cadaveric material—The results are shown in Table I. All sections showed the typical mosaic pattern of normal muscle, arising from the intermingling of muscle fibres from individual motor units (see Fig. 1). Low ATPase

older group of eleven subjects aged thirty-five to fifty-eight. The histochemical values were then averaged for the two groups, and the differences and their significance were calculated.

FIG. 1

FIG. 2

FIGS. 1 AND 2
Sections of multifidus showing a normal mosaic distribution of Low and High ATPase fibres with normal polygonal outline (High ATPase—dark staining). The histograms show the distribution of areas of the two fibre types, both being within normal limits. In Figure 1 the section ($\times 240$) is from the L.5 level in subject C.1 (see Table I). In Figure 2 the section ($\times 50$) is from the L.3/4 level in patient L.1 (see unpublished table).

FIG. 3

Fascicles containing predominantly Low ATPase fibres in a man of 58 with root signs. The section ($\times 50$) is from the L.5/S.1 level in patient L.17 (see unpublished table).

The principal finding was that the Low/High mean-area-ratio was greater in the older group (Fig. 5):

younger: 1.27 (s.d. 0.18);
older: 2.21 (s.d. 0.41) [$p < 0.001$].

This arose mainly from a fall in the cross-sectional area of High ATPase fibres:

younger: 3,457 μ^2 (s.d. 920);
older: 2,239 μ^2 (s.d. 1,010) [$p < 0.01$]

FIG. 4

Showing angulation of the ATPase fibres, which are diminished in number, from a patient with root signs. The histograms show that most of the High ATPase fibres are less than 1,000 μ^2 . The section ($\times 200$) is from the L3/4 level in patient L.14 (see unpublished table).

but there was also a greater variation in the size of High ATPase fibres with age, shown by an increased coefficient of variation:

younger: 26.7 per cent (s.d. 6.3);
older: 41.3 per cent (s.d. 11.4) [$p < 0.001$].

Lumbar flexibility

In the fourteen patients on whom measurements of the lumbar curve were made in flexed and extended postures, there were six who could flex to a forward curve of between 20 and 40 degrees, and eight with limited flexion who could only reach curves between -5 and 15 degrees. In the latter group the Low/High mean-area-ratio was greater:

full flexion: 1.3 (s.d. 0.3);
reduced flexion: 2.3 (s.d. 0.8) [$p < 0.001$].

Though the cross-sectional areas of the Low ATPase fibres were somewhat greater in those with limited flexions, the difference was not significant. But in calculating the coefficient of correlation between the degree of maximal forward flexion and the mean areas of Low ATPase fibres for individuals, the coefficient ($r = -0.53$) showed a significant inverse relationship ($p < 0.05$). There was a similar inverse relationship with Low ATPase mean areas ($r = -0.66$, $p < 0.01$) when related to the total range of movement between maximally flexed and extended postures. Thus limitation of lumbar flexibility was associated with relatively larger Low ATPase fibres. The other finding with respect to the maximal flexed lumbar curve was that the variation in size was significantly greater in those with limited forward curves:

Coefficients of variation of mean fibre areas

	Low ATPase	High ATPase
full flexion:	23.7 per cent (s.d. 2.7)	27.3 per cent (s.d. 5.1)
reduced flexion:	35.0 per cent (s.d. 10.6) [$p < 0.02$]	38.3 per cent (s.d. 7.6) [$p < 0.01$].

FIG. 5

Showing diminution in size and proportion of the High ATPase fibres, typical of specimens from older patients and with limited spinal flexion. The histogram shows marked variation in the cross-sectional area of the Low ATPase fibres, with reduction in size of the High ATPase fibres. The section ($\times 240$) is from the L5/S1 level in patient L.16 (see unpublished table).

Root signs

Of the seventeen patients, nine had positive signs of root involvement pre-operatively and eight did not, the mean ages of the two groups being 36.6 years (range nineteen to fifty-eight) and 33.1 years (range fifteen to fifty-five) respectively. High ATPase fibres formed only 27 per cent of the total in those with positive root signs, but 40 per cent in those without ($p < 0.05$). In sections from six patients whole fascicles were seen with no High ATPase fibres. Four of these patients (L.2, L.3, L.12 and L.17) had positive signs but the other two did not (L.10, a patient with a previous history of surgery for disc prolapse, and L.11).

High ATPase fibres tended to be somewhat larger and more varied in cross-sectional area in those with positive root signs [$2,811 \mu^2$ (s.d. 1,061)], when compared with those from patients without [$2,122 \mu^2$ (s.d. 649)], though the difference was not significant ($p < 0.10$). In three patients with positive signs (L.14, L.15 and L.16) the variation in size was marked by the presence of a large proportion of High ATPase fibres less than $1,000 \mu^2$ in area and the polygonal outline of the High ATPase fibres was more angular (see Fig. 4). Fibres of less than $1,000 \mu^2$ in cross-sectional area and with angular outlines were not seen in the absence of root signs.

Lumbar instability

The patients were divided into two groups according to the presence or absence of the "extension catch" (a sign of lumbar spinal instability) and the histochemical results were compared. No differences, however, emerged from this analysis.

DISCUSSION

The technique used in these experiments to differentiate the fibres with fast-twitch response from those with slow on the basis of the activity of myosin ATPase is well established (Robbins, Karpati and Engel 1969; Syrový and Gutmann 1970; Burke *et al.* 1971). Fibres high in myosin ATPase activity will therefore be referred to as Fast and those with low myosin ATPase activity as Slow fibres in the discussion which follows.

In the cadaveric specimens and in many of the biopsies, the appearance of the Fast and Slow fibres presented the mosaic pattern typical of that seen in healthy muscles of the limbs, but there is a problem when interpreting the meaning of deviations from the normal. Unfortunately back pain and stiffness are so widespread that normality is not assured even in material from supposedly healthy subjects killed in accidents. There is no simple way of obtaining a comparable series of results from subjects known to be free of spinal symptoms and pathology. In this series of observations, however, there have been a number of significant features. These concern the effects of age, the changes associated with loss of spinal mobility, the changes associated with clinical signs

of root pathology, and finally, the effects of neural influence on fibre-type.

Changes with age—In this series the major and statistically significant change with age was the associated increase in the ratio of Slow/Fast fibre size. This arose largely from a fall in the size of Fast fibres with age, and to some extent from the less marked tendency for Slow fibres to become bigger. There was also a tendency for the proportion of Slow fibres to increase, but on the numbers examined this was not significant. The trend nevertheless is comparable with the results of Jennekens, Tomlinson and Walton (1971). The other major change with age was the increased variation in size of fibres, especially of the Fast fibres, and similar findings were again noted by Jennekens *et al.* (1971). They, however, reported the occurrence in old age of markedly atrophic fibres and, furthermore, of a selection of structural changes including target fibres, pseudo-cores and other intracellular irregularities, none of which were seen in multifidus. These results suggest that with ageing, multifidus adopts an increasingly postural role as Slow fibres predominate.

Changes with limited lumbar flexibility—Although the two measures of flexibility were not significantly related to the ages of the patients, the histochemical effects were similar, particularly with age and limited lumbar flexion. Increase in the ratio of Slow/Fast fibre-size was an associated factor common to both; it suggests a transition to more tonic function and a reduced capacity for rapid, phasic activity.

Changes associated with signs of root involvement—The patients with and without positive root signs were well matched for age. The proportion of Slow fibres as a percentage of the total increased, and the cross-sectional areas of Fast fibres were somewhat greater in those with positive root signs. The difference between the results for the two groups was quite distinct from the changes associated with age. Yet Jennekens *et al.* (1971, 1972) reported changes with age which they attributed to denervation and reinnervation. They saw groups of the two types of fibre clumped so that the mosaic pattern was lost. Only in deltoid did they report whole fascicles of Slow fibres comparable to those reported here.

The grouping of fibre-types is said to arise from terminal collateral branching from an intact motoneurone to supply neighbouring muscle fibres whose motoneurone is damaged or cut (Kugelberg *et al.* 1970). When grouping, as seen here, is only of Slow fibres, it suggests that loss of conductivity affects Fast motoneurones alone. This would account for the falls in the proportion of Fast fibres, and for the occurrence of small, angular, probably atrophied Fast fibres seen in patients with signs of root involvement. It is consistent with the clinical impression that such denervation as occurs in lumbar and sacral root lesions, is selective, and with the fact that different types of fibre in peripheral nerves vary in their sensitivity to ischaemic compression or other mechanical stimuli (Marshall 1952; Lindquist, Nilsson and Skoglund 1973).

Neural influences on fibre-type—The possibility remains that changes in the histochemical pattern may take place in the absence of loss of neural conductivity, for with age and in the presence of symptoms spinal movements are reduced in range and velocity. In other words, fibre-type may be influenced by a consistent change in the temporal pattern of activity in motoneurons, and not only by neuropathological change. In idiopathic scoliosis, for example, Fidler *et al.* (1974) showed that the proportion of Slow fibres was consistently greater on the convex side at the apex of the curve, where tonic activity is a typical finding (Riddle and Roaf 1955, Jansen 1960, Žuk 1962, Horn 1969).

There is evidence from experiments in which Slow and Fast muscle have been cross-innervated that reciprocal changes in mechanical responses can be induced

(Bárány and Close 1971). More significantly, the decrease in contraction time of Slow rabbit muscle following tenotomy or section of the cord can be prevented by stimulation at 10 Hz, but not at 40 Hz (Salmons and Vrbová 1969), while prolonged stimulation of Fast rabbit muscle at 10 Hz produces an increase in contraction time and a reduction in glycolytic activity (Pette, Staudte and Vrbová 1972). Thus low frequency stimulation had effects comparable to those induced by innervating a Fast muscle with nerves formerly supplying Slow muscle (Close 1971), and it can be assumed that the metabolism of the cell and its enzymic activity can be modified neurogenically. But this leaves open the question of whether, due to a change in activity at high level, there can be a transition in a given motoneurone from a Fast to a Slow pattern of discharge in man.

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