MOTOR INNERVATION AND FIBER TYPE PATTERN IN AMYOTROPHIC LATERAL SCLEROSIS AND IN CHARCOT-MARIE-TOOTH DISEASE

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Reprinted from MUSCLE & NERVE March/April 78

HM/MD Houghton Mifflin Professional Publishers
Two Park St., Boston, Massachusetts 02107

A quantitive study of the terminal innervation ratio (TIR) was conducted using 18 amyotrophic lateral sclerosis (ALS) and 12 Charcot-Marie-Tooth disease (CMT) muscle biopsies. Morphometric and histochemical analyses of muscle fibers were performed in 9 ALS and 6 CMT biopsies. The results revealed that TIR and type grouping were significantly greater in CMT than in ALS. The proportion of type 3 fibers was higher in ALS, though the proportion of intermediate and type 0 fibers was significantly higher in CMT. The atrophy factor was significantly greater in type 3 than in types 1 and 2 fibers, but it was not significantly different in type 0 and intermediate fibers as compared to types 1 and 2. It appears, therefore, that CMT has a better capacity for collateral reinnervation than ALS. Type 0 and intermediate fibers may represent altered endproducts of successful collateral reinnervation.

MUSCLE & NERVE 1:133–139 1978

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Histochemical studies of denervated skeletal muscles have disclosed a characteristic change in the distribution of the motor units, the so-called type grouping[10] that probably results from the reinnervation of adjacent denervated fibers by collateral sprouting from healthy motoneurons.[12] It may be assumed from these findings that the efficiency of the compensatory process of collateral reinnervation and the reactive muscle-fiber-type pattern varies according to the severity of the disease process.

The purpose of the present study was to compare the motor innervation and the muscle-fiber-type pattern in two kinds of progressive muscular atrophy: (1) amyotrophic lateral sclerosis (ALS) and (2) Charcot-Marie-Tooth disease (CMT), the latter having a much slower and milder evolution than the former.

MATERIALS AND METHODS

Muscle biopsies were obtained from 21 patients with ALS and from 16 patients with CMT. The muscles biopsied were palmaris longus, flexor carpi radialis, or vastus medialis. Whenever possible, we chose for biopsy muscles that appeared clinically normal or had only a slight degree of atrophy and weakness.

All biopsies were vitally stained with methylene blue after electrical localization of the terminal innervation area.[4,5] A quantitative estimation of the collateral branching of motor axons was performed in 18 biopsies from ALS patients and in 12 biopsies from CMT patients, using as a measurement the terminal innervation ratio (TIR)—that is, the number of muscle fibers innervated by one subterminal axon.[5,6]

The morphometric analysis of muscle fiber types was performed in nine ALS biopsies and in six CMT biopsies. Transverse frozen sections (liquid-nitrogen–cooled isopentane, –170 C) were cut with a cryostat and stained for myosin ATPase[2,15] and NADH diaphorase.[14] The analyses were performed on 200 to 300 muscle fibers in each sample. In two of the CMT biopsies, as a result of the marked variability of the fiber-type pattern, the quantitative data were collected in four fields in one biopsy (800 fibers) and in two fields in the other (400 fibers) to increase the accuracy of the measurements. Thus, 10 samples were analyzed in CMT. The mean diameter of the muscle fibers and the standard deviation (SD) were estimated, and the proportions of type 1 and type 2 fibers were recorded. The degree of atrophy was estimated by the atrophy factor (AF) of Brooke and Engel.[1] Fibers having no reciprocal

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This work was supported by grants from the FRSM of Belgium and the Free University of Brussels. The authors wish to thank Mr. J. Durdu and Mrs. G. Lousberg for technical help.

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Received for publication September 7, 1977.

relationship between the two enzymes were labeled either type 3, when they had a high activity for both enzymes, or intermediate, when they had a medium enzymatic activity for ATPase as compared to typical type 1 and type 2 fibers.[17,18] Fibers having a low activity for both enzymes were labeled type 0. The proportions of type 3, type 0, and intermediate fibers were recorded. The type grouping was estimated on the basis of the greatest number of adjacent and enclosed fibers of the same type found in the specimen.[9]

The quantitative data obtained were compared between ALS and CMT as well as with the control values obtained from biopsies from patients without clinical or histological evidence of neuromuscular disorder. Controls were obtained from 56 biopsies for TIR[6] and 35 biopsies for fiber types. Means and SDs were compared using the Student t test.

RESULTS

The morphometric data on the muscle fibers are reported in tables 1 and 2. The atrophy factors were significantly increased to the same extent in both diseases. A highly significant reduction in the number of type 2 fibers was found in CMT. Muscle fibers with aberrant enzymatic profiles are scarce in normal muscles. The proportions of type 3 and intermediate fibers were significantly higher than the controls in both ALS and CMT. Type 0 fibers were observed only once, in a biopsy from CMT, in which they represented 56% of the fiber population (figs. 1 and 2). Type 3 fibers were usually distributed at random and were often small and angulated (figs. 1, 3, and 4), and their AF was significantly higher than those of fiber types 1 and 2 (table 1). Intermediate fibers, also randomly distributed in ALS, tended to be grouped in CMT (fig. 3). Type 0 fibers seen in one biopsy from CMT were also grouped in large bundles (figs. 1 and 2). The AFs of type 0 and intermediate fibers were not significantly different from the AFs of types 1 and 2 (table 1). Comparison of the means suggested a reciprocal relationship between the proportions of type 3 fibers and of intermediate fibers, the former being greater in ALS and the latter being greater in CMT. However, because of the marked dispersion of individual values, the difference was not statistically significant. Nevertheless, when we considered intermediate and type 0 fibers together, the difference between ALS and CMT was statistically significant at the .05 level.

The TIR was abnormally high in both ALS and CMT (fig. 5), but its mean value was significantly higher in CMT (table 3). Type grouping was less conspicuous in ALS (fig. 4) than in CMT (figs. 1–3), and the proportions of contiguous and enclosed fibers of both types were greater in CMT (fig. 6). Statistical comparison of the means showed that the differences were significant (table 3). Comparison of type grouping between the ALS and control patients disclosed a significant difference for type 2 fibers only, whereas this difference was highly significant for both type 1 and type 2 fibers in CMT (table 3).

DISCUSSION

It has already been noted that type grouping is more frequent in CMT than in ALS.[7,8] The present study provides statistical evidence of this difference in motor unit distribution for these two diseases. In addition, we

Table 1. Atrophy factor (AF).

Sample		Type 1 mean	Type 1 SD	Type 2 mean	Type 2 SD	Types 1 + 2 mean	Types 1 + 2 SD	Type 3 mean	Type 3 SD	Intermediate (i) mean	Intermediate (i) SD	Type 0 mean	Type 0 SD
Controls		76	175	124	153	100	165	—	—	—	—	—	—
ALS		365	462	495	638	430	544	1151	992	925	810	—	—
CMT		294	405	617	676	447	559	1220	1032	490	1094	0	0
Comparison controls and ALS	t	3.012		4.057		—	—	—	—	—	—	—	—
	p	<.005		<.001		—	—	—	—	—	—	—	—
Comparison controls and CMT	t	2.511		2.923		—	—	—	—	—	—	—	—
	p	<.02		<.001		—	—	—	—	—	—	—	—
Comparison ALS and CMT	t	0.356		0.396		0.095		0.562		0.883			
	p	NS[a]		NS		NS		NS		NS			

		Comparison AF_3 and AF_{1+2} in ALS	Comparison AF_3 and AF_{1+2} in CMT	Comparison AF_i and AF_{1+2} in ALS	Comparison AF_i and AF_{1+2} in CMT	Comparison AF_{i+0} and AF_3 in ALS and CMT
	t	2.457	2.534	1.890	0.132	2.313
	p	<.025	<.02	NS	NS	<.05

[a] *NS = not statistically significant.*

Table 2. Proportion of fiber types.

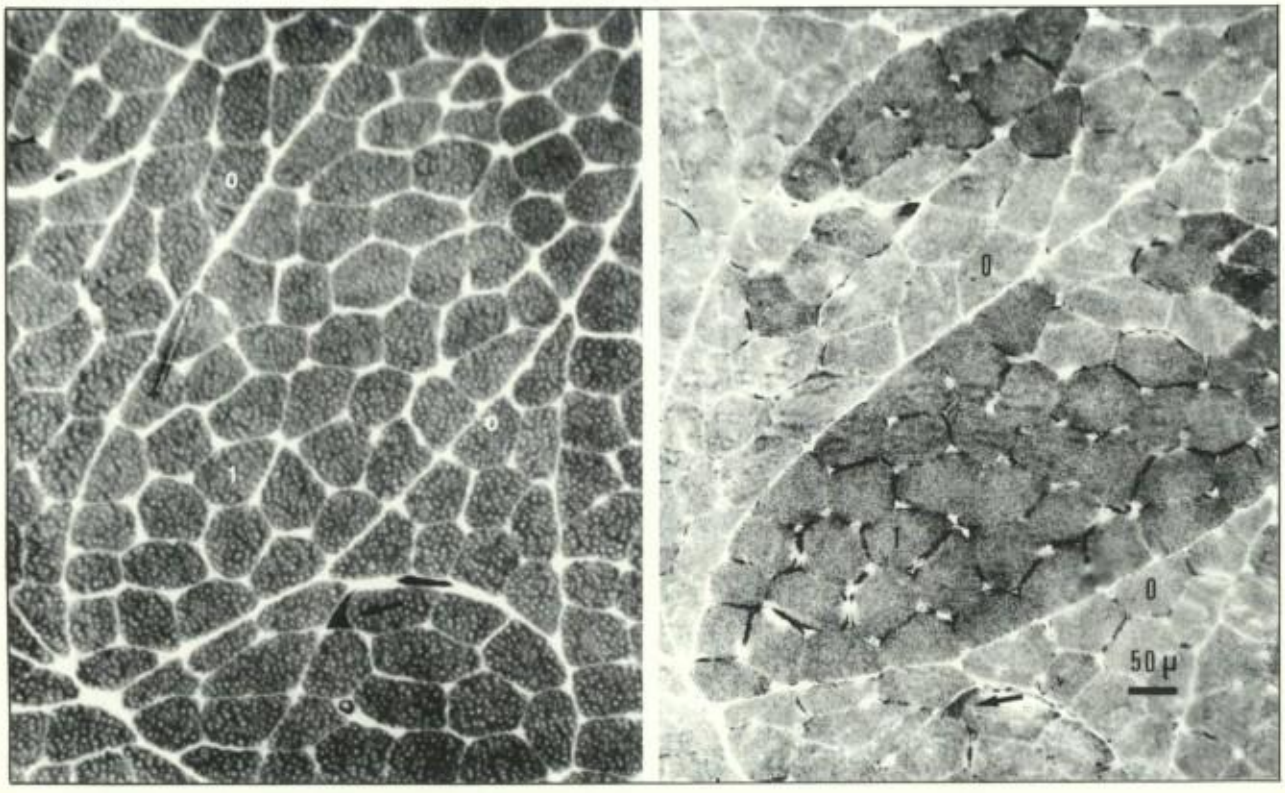
Sample		Type 1 (%) mean	Type 1 (%) SD	Type 2 (%) mean	Type 2 (%) SD	Type 3 (%) mean	Type 3 (%) SD	Intermediate (%) mean	Intermediate (%) SD	Type 0 (%) mean	Type 0 (%) SD	Intermediate + type 0 (%) mean	Intermediate + type 0 (%) SD
Controls		47.3	9.5	55.1	9.4	0.4	0.8	0.3	0.5	0.03	0.1	–	–
ALS		36.1	15.8	45.5	21.9	14.9	12.4	3.5	2.8	0	0	3.5	2.8
CMT		36.7	32.8	34.2	32.3	4.9	11.2	12.7	23.9	11.4	24.3	24.1	29.6
Comparison controls and ALS	t	1.870		2.004		7.101		5.480					
	p	NS[a]		NS		<.001		<.001					
Comparison controls and CMT	t	1.712		3.431		2.454		3.152					
	p	<.01		<.005		<.02		<.005					
Comparison ALS and CMT	t	0.053		0.881		1.866		1.142				2.07	
	p	NS		NS		NS		NS				<.05	

[a] *NS = not statistically significant.*

have established that the process of collateral ramification is more marked in CMT than in ALS, with TIR significantly higher in the former. These differences suggest that the unaffected motoneurons in CMT have a better capacity for reinnervation of adjacent denervated muscle fibers than their counterparts in ALS. Electromyographic estimation of fiber density[16] has produced results in agreement with the morphometric data reported here. The greater efficiency of the unaffected motoneurons in CMT to compensate for the loss of motor units can be related to the slow and sometimes mild evolution of CMT as compared to ALS.

In addition, the coexistence of increased TIR and increased degree of type grouping as shown by this study provides evidence that both changes are the

Figure 1. CMT biopsy no. 1261. Serial sections: left, myosin ATPase; right, NADH diaphorase. Type 1 grouping (1); numerous grouped type 0 fibers (0); type 3 fiber (arrow).



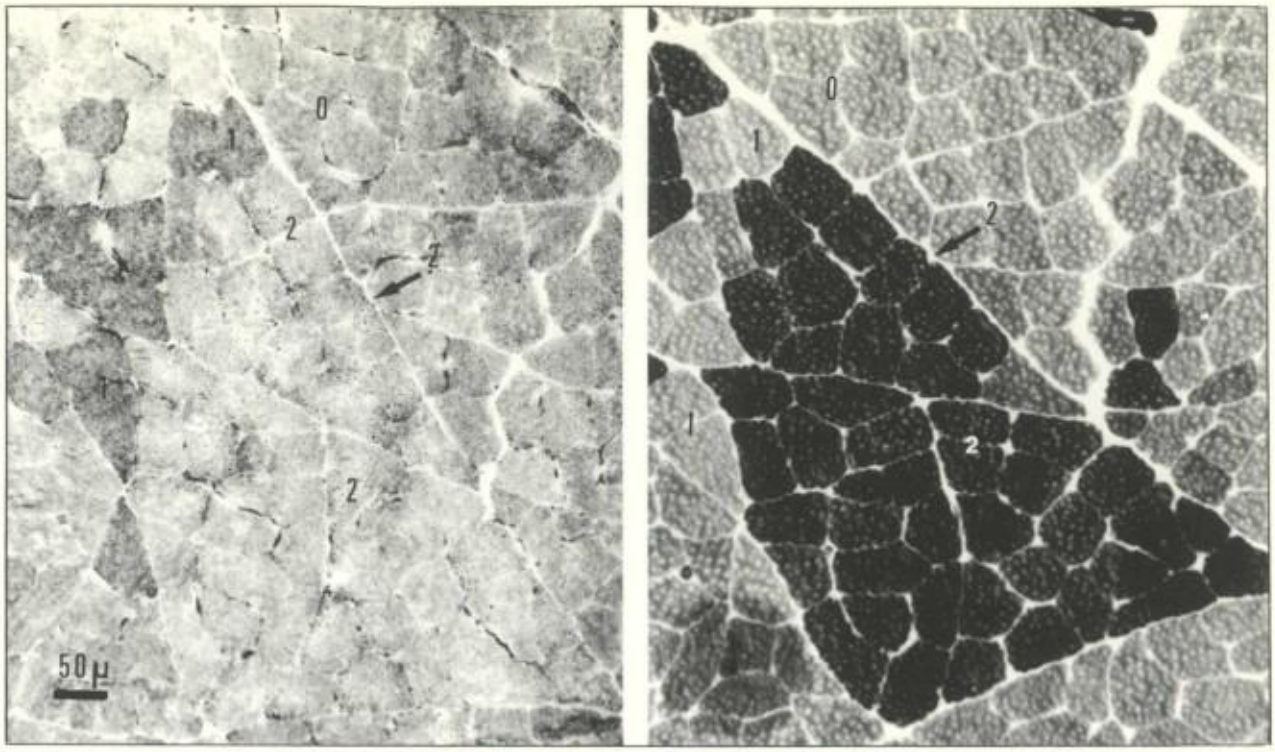


Figure 2. CMT biopsy no. 1261. Serial sections: left, NADH diaphorase; right, myosin ATPase. Type 2 grouping (2); type 1 fibers (1); grouped type 0 fibers (0).

result of the compensatory increase in the size of residual motor units that takes place in chronic denervation. Similarly, the formation of muscle fiber groups having the same enzymatic profile, typical of either type 1 or type 2 fibers, suggests that reinnervation of muscle fibers by collaterals from motoneurons of a different type may lead to full conversion of their metabolic characteristics. This has been demonstrated by experimental cross-reinnervation.[1]

In this respect, it has been assumed that in neurogenic conditions, muscle fibers which exhibit a blurring of the usual reciprocal relationship between myosin ATPase and NADH diaphorase represent a transitional state in the process of enzymatic conversion.[8,13,17,18] Various patterns of aberrant enzymatic profiles have been described: muscle fibers with intermediate NADH diaphorase activity,[13] or with a strong reactivity for both myosin ATPase and NADH di-

Table 3. TIR and type grouping.

		TIR		Contiguous fibers				Enclosed fibers			
				Type 1		Type 2		Type 1		Type 2	
Sample		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Controls		1.11	0.05	10.70	2.90	11.6	4.00	0.60	0.90	1.50	1.90
ALS		1.66	0.30	10.70	7.50	18.0	7.60	1.40	2.70	4.50	4.40
CMT		2.16	0.56	74.17	68.30	62.5	60.50	63.30	72.00	46.00	59.70
Comparison controls and ALS	t	12.700		0.994		2.893		1.407		3.122	
	p	<.001		NS[a]		<.01		NS		<.005	
Comparison controls and CMT	t	14.300		5.838		5.236		5.502		6.325	
	p	<.001		<.001		<.001		<.001		<.001	
Comparison ALS and CMT	t	3.222		2.816		2.221		2.628		2.117	
	p	<.005		<.05		<.05		<.025		<.05	

[a]*NS = not statistically significant.*

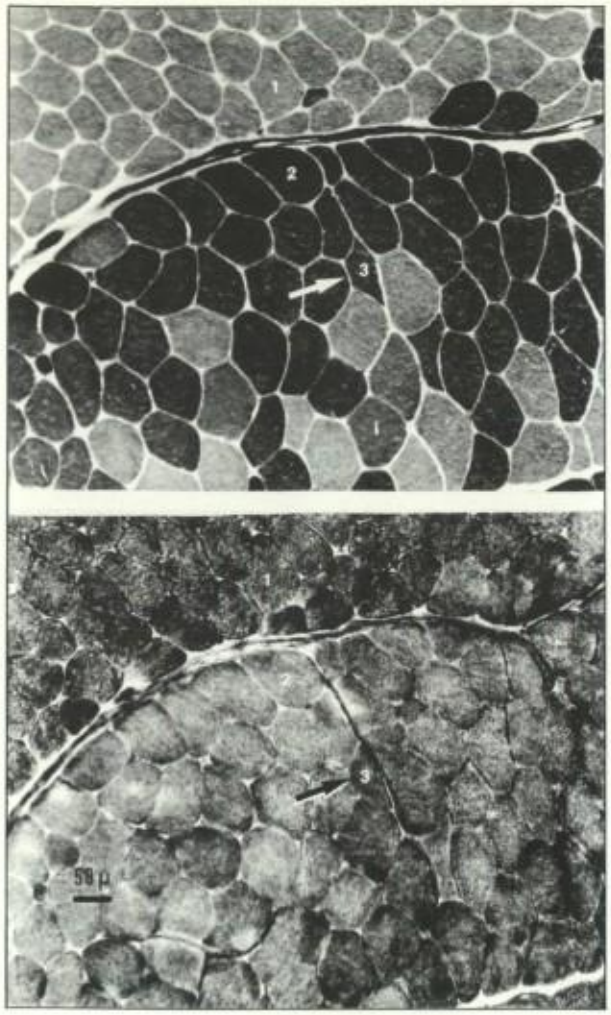


Figure 3. CMT biopsy no. 1149. Serial sections: top, myosin ATPase; bottom, NADH diaphorase. Type 3 fiber (arrow); type 1 grouping (1); type 2 grouping (2); intermediate fiber (i).

aphorase (type 3 fibers, according to Telerman-Toppet and Coërs),[17] or with low myosin ATPase and low SDH activity.[11] Jennekens et al[8] also described "hybrid" fibers with low myosin ATPase and low capacity for aerobic metabolism. In the present study these "hybrid" fibers were labeled type 0. Other aberrant fibers having an intermediate enzymatic activity for ATPase activity, as compared to typical type 1 or type 2 fibers, were placed in a loosely defined group of intermediate fibers.

Grouped type 0 fibers were found only once, in a case of CMT. Intermediate fibers were most numerous in CMT, in which they also tended to be grouped. Conversely, the proportion of type 3 fibers was higher in ALS. Type 3 fibers were randomly distributed and often small and angulated, and their AF was significantly higher than that of type 1, type 2, or intermediate fibers. Type 0 fibers had a normal size. The AF of intermediate fibers was not significantly different from that of type 1 or type 2 fibers.

These differences in distribution and size suggest

Figure 4. ALS biopsy no. 1090. Serial sections: left, myosin ATPase; right, NADH diaphorase. Grouping of type 1 (1) and type 2 (2) fibers is not conspicuous. Small type 3 fibers (arrow) and intermediate fibers (i).

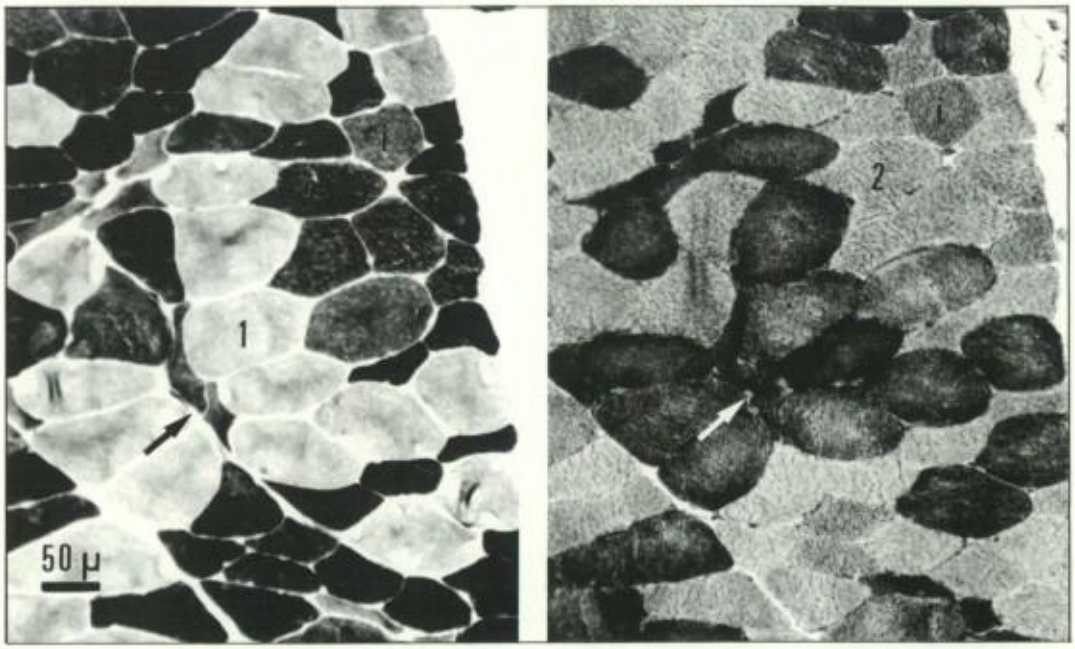
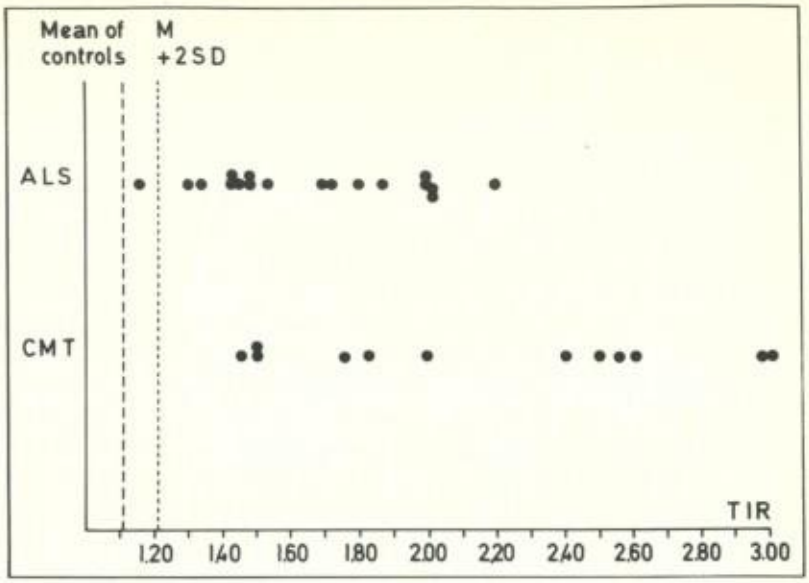


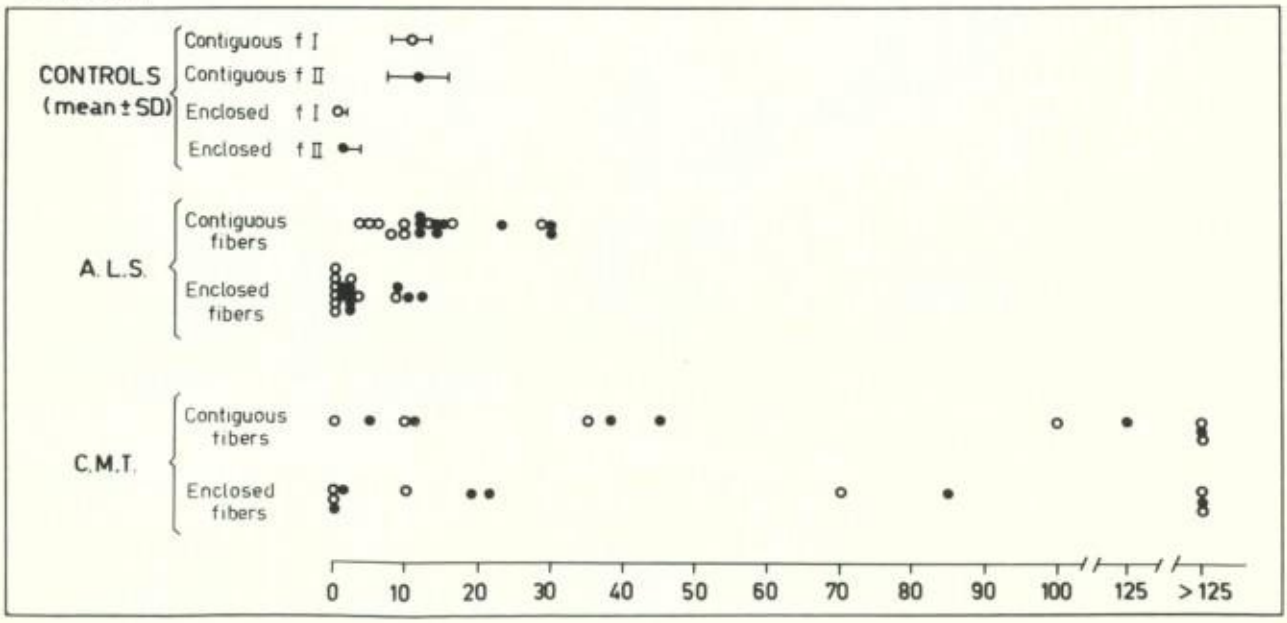
Figure 5. Distribution of TIR in ALS and CMT.



that type 0 and intermediate fibers do not have the same functional significance as type 3 fibers. It may be assumed that type 3 fibers either are in a dynamic process of conversion during reinnervation by collaterals from a motoneuron of the other type, or are undergoing denervation after failure of reinnervation. Type 0 and intermediate fibers, significantly larger than type 3 fibers, probably represent a steady state of incomplete enzymatic conversion after successful collateral reinnervation, as suggested by Jennekens et al.[8]

Such "hybrid" fibers (type 0 and intermediate), together with type grouping, were also found in Kugelberg-Welander disease[11] and in infantile benign spinal muscular atrophy.[8] Therefore, it seems likely that the differences in motor innervation and fiber-type pattern observed in ALS and CMT are related primarily to differences in the severity and time course of the disease processes, and not to the differences in target structures, that is, motoneuron or peripheral nerves.

Figure 6. Distribution of contiguous and enclosed type 1 (open circles) and type 2 (closed circles) fibers in ALS and CMT.



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