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Fibre Hybrids in Type Groups

An Investigation of Human Muscle Biopsies

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INTRODUCTION

Type grouping is frequently observed in muscle biopsies from patients with lower motor neurone disease or peripheral neuropathies. It is considered to be due to reinnervation (Engel 1970). Though reinnervation may lead to full conversion of the metabolic characteristics of muscle fibres (Close 1972), several authors have observed unusual histochemical characteristics of muscle fibres in type groups. Mastaglia and Walton (1971) described fibre hybrids with low myosin ATPase and low SDH activity in muscle biopsies from 5 patients with Kugelberg–Welander's syndrome. Previously Dubowitz (1968) had observed fibres with low myosin ATPase activity and a high level of phosphorylase and NADHTR activity in a case of Werdnig–Hoffman's disease. Brooke, Williamson and Kaiser (1971) noticed muscle fibres showing discrepancies between myosin ATPase and NADHTR activity in fibre type groups in reinnervation experiments. These authors pointed out that they had seen similar discrepancies in muscle biopsies from patients with denervating diseases. Robbins, Karpati and Engel (1969) noticed in cross-reinnervated animal muscle numerous muscle fibres with intermediate activity of several enzymes; only the myosin ATPase activity showed better preservation of fibre types.

Morris and Woolf (1970) examined intramuscular nerves in biopsies from patients with the Landry–Guillain–Barré syndrome. Muscle fibres of different types were sometimes seen to be innervated by collaterals of the same axon. Increase of the terminal innervation ratio and increase of the number of muscle fibres with intermediate NADHTR activity were seen by Morris (1971) in neurogenic muscle disease. He suggested that these muscle fibres had been reinnervated but had not yet completed the change from one type to another. This hypothesis was generally accepted as a plausible explanation for deviant histochemical features of reinnervated muscle fibres, parti-

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cularly in so far as intermediate enzyme activity was concerned. It is, however, questionable whether the presence of fibre hybrids of the type described by Mastaglia and Walton and by Dubowitz is equally well explainable by this hypothesis. This would be possible only if one assumed that changes in the capacities of aerobic and anaerobic metabolism could develop at a markedly different pace.

The purpose of the present study was to evaluate the validity of the conversion hypothesis on presumably reinnervated muscle fibres in human neurogenic muscle disease. It was assumed that at least a number of muscle fibres in type groups of non-atrophic fibres were reinnervated. In serial or adjacent sections of muscle biopsies parameters of aerobic metabolism and of glycolytic metabolism were compared to myosin ATPase activity.

MATERIAL AND METHODS

Forty-two biopsies had been taken from patients with progressive or chronic lower motor neurone disease or with peripheral neuropathy. The muscle tissue blocks had been frozen in isopentane which had been cooled in liquid nitrogen. Transverse serial or adjacent sections had been used for histochemistry and for routine histological staining. The activity of myosin ATPase (adenosine triphosphatase, EC 3.6.1.3) had been determined at pH 9.4 (Padykula and Herman 1955) or according to Meijer's method (1970). NADHTR (nicotinamide adenine dinucleotide: tetrazolium oxidoreductase, EC 1.6.99.2, Burstone 1962; Barka and Anderson 1963) and SDH (succinate dehydrogenase, EC 1.3.99.1, Nachlas, Tsou, Souza, Cheng and De Seligman 1957) activity had been used as parameters of aerobic metabolism. The glycogen content (PAS technique combined with diastase digestion) and glucan phosphorylase (EC 2.4.1.1, Meijer 1968) and GPOx L-glycerol-3-phosphate: menadione oxidoreductase (EC 1.1.99.5, Wattenberg and Leong 1960) activity had been used as parameters of glycolytic metabolism. The method used for determination of phosphorylase activity was independent of muscle fibre glycogen content.

Myosin ATPase activities were used as the criterion for histochemical typing. Muscle fibres were considered atrophic when their shortest diameter was less than 20 μm. Fibre type grouping was assessed by counting enclosed fibres as outlined by Johnson, Polgar, Weightman and Appleton (1973). A muscle fibre was considered to be enclosed if it was surrounded by fibres of its own histochemical type (Jennekens, Tomlinson and Walton 1971). The distribution of atrophic fibres was described as disseminated when atrophic fibres were spread between non-atrophic fibres and when less than 10 atrophic fibres were lying together. When most fibres were atrophic and only a few non-atrophic fibres were spread amongst the atrophic fibres, this was considered to be a final phase in disseminated fibre atrophy. Groups of more than 10 and less than 80 atrophic fibres were called "small". Groups of more than 80 atrophic fibres were called "large".

RESULTS

Type grouping was found in 27 biopsies. The presence of type grouping was usually

TABLE I

HISTOCHEMICAL ASPECTS OF FIBRES IN MYOSIN ATPASE UNIFORM GROUPS IN BIOPSIES WITH FIBRE TYPE PREDOMINANCE

No.	*Sex*	*Age (yrs)*	*Duration illness (yrs)*	*Diagnosis*[a]	*Muscle*	*Distribution atrophic fibres*	*Myosin ATPase*	*NADHTR*[b]	*SDH*[b]	*GPOx*[b]	*Phosphorylase*[b]	*Glycogen*[b]
Ch 2288	♀	76	2	polyneuropathy (unknown cause)	tib. ant.	large groups	type I	uniform	uniform	uniform	uniform	uniform
Ch 2338	♂	63	6	p s m a	deltoid	large groups	type I	h	h	h and l	h and l	h and l
Ch 2430	♂	48	40	b s m a	tib. ant.	large groups	type I	h	h	h and l	h and l	h and l
Ch 3942	♀	47	37	C–M–T	quadriceps	large groups	type I	h	h	h and l	h and l	h and l
H 102	♂	48	26	b s m a	tib. ant.	large groups	type I	l		l and i	l and h	l and h
H 107	♀	72	5	polyneuropathy (unknown cause)	tib. ant.	large groups	type I	h	–	h and l	h and l	h and l
H 125	♂	6	$5\frac{1}{2}$	b i s m a	biceps brachii	large groups	type II	l and i	–	h and i	h	h and i

[a] p s m a = progressive spinal muscular atrophy (Aran–Duchenne).
b s m a = benign infantile spinal muscular atrophy
C M–T = Charcot–Marie-Tooth.
b i s m a = benign infantile spinal muscular atrophy.
a l s = amyotrophic lateral sclerosis (see Table 2).

[b] h = high activity/level.
i = intermediate activity/level.
l = low activity/level.

Fig. 1. Case H107, ×80. Serial sections: *A*: myosin ATPase, low in almost all fibres; *B*: NADHTR, activity is nearly uniform; *C*: GPOx, preponderance of fibres with low activity, high activity in a fair number of fibres; *D*: phosphorylase, fibres with low, high and intermediate activity; *E*: PAS, fibres with high and low glycogen content.

related to the presence of small or large groups of atrophic fibres. Large groups of atrophic fibres were not seen in biopsies without type grouping; in these cases atrophic fibres were usually disseminated (Tables 1, 2, 3 and 4). There was no obvious relation between the kind of disorder of the peripheral motor neurones and the occurrence of type grouping, but probably there was some relation to the duration of illness. Type grouping was found in most cases of the Charcot–Marie–Tooth syndrome and in benign spinal muscular atrophy but it was often absent in amyotrophic lateral sclerosis and in progressive spinal muscular atrophy.

A. Histochemically Uniform Non-atrophic Groups of Muscle Fibres

Biopsies with type grouping were subdivided into 3 categories.

(1) Fibre type predominance (Table 1)

In 7 cases nearly all muscle fibres belonged to one type. In 6 biopsies nearly all non-atrophic fibres showed type I myosin ATPase activity, in 1 case nearly all non-atrophic fibres were classified as type II. Few fibres showed intermediate ATPase activity. In cases with type I predominance histochemical uniformity was also well expressed by the parameters of aerobic metabolism, which showed an intermediate or high activity. In 1 case the type I fibres, which were of large size, had intermediate or low NADHTR activity.

Histochemical uniformity was less well expressed by the parameters of glycolytic metabolism. In 5 of 6 biopsies 3–25% of myosin ATPase type I fibres showed a high or intermediate phosphorylase and GPOx activity and a high or intermediate glycogen content (Figs. 1 and 2). There were some discrepancies between these 3 parameters: a high or intermediate glycogen content and a high or intermediate phosphorylase and GPOx activity were not present in equal numbers of non-atrophic fibres.

The one case with type II fibre predominance showed some variation of enzyme activity and glycogen content within and between fascicles, the exception being the phosphorylase activity which was uniform in all non-atrophic fibres.

(2) Biopsies with type I or type II groups (Table 2)

In 11 biopsies type I and (or) type II groups were seen, fibres of both types being present in all fields. In 9 of these biopsies the non-atrophic fibres were partly arranged in type groups and partly in a mosaic or a mosaic-like pattern. In 2 biopsies all non-atrophic fibres were arranged in type groups. Sometimes whole fascicles showed uniform enzyme activity, differences in enzyme activity being observed between the fascicles. This arrangement was considered to be due to the fact that axonal sprouts of intramuscular nerve fibres are unable to cross the perimysial connective tissue barrier (Kugelberg, Edström and Abruzzese 1970).

Myosin ATPase activity was usually very different in type I and type II fibres: fibres with intermediate activity were few in number. NADHTR and SDH activities were usually reciprocal to myosin ATPase activity, though intermediate activity occurred more frequently than in the myosin ATPase stain. With minor variations between single muscle fibres the histochemical uniformity was well expressed by

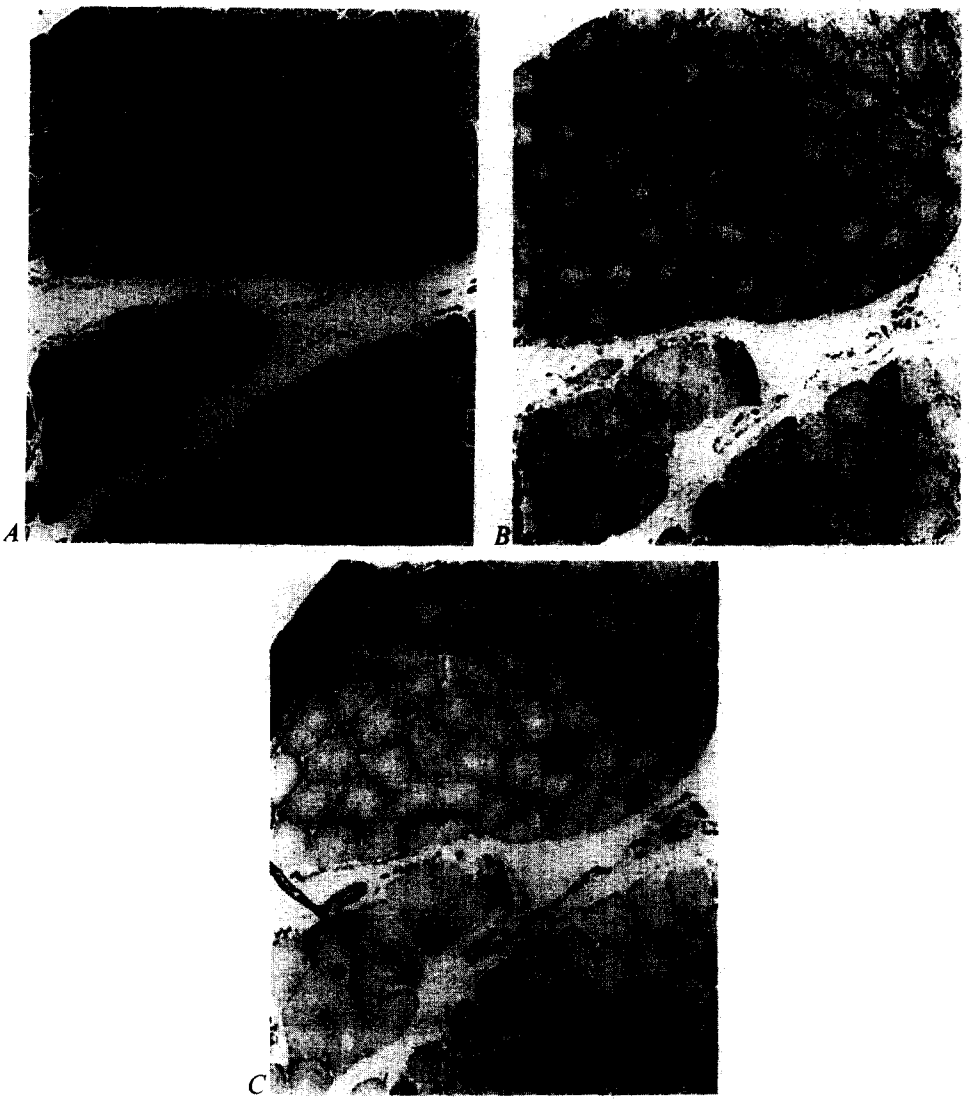

Fig. 2. Case Ch 2430, × 120. Serial sections. *A*: myosin ATPase; *B*: SDH; *C*: GPOx.

these enzymes. Though relatively small groups were sometimes difficult to localize in successive sections, it was our impression that type grouping was also expressed by the parameters of glycolytic metabolism. In at least 5 biopsies a small number (less than 5%) of myosin ATPase type I fibres showed intermediate or high capacity of glycolytic metabolism.

(3) Myosin ATPase uniform groups (Table 3)

In 9 cases myosin ATPase activity was almost or completely uniform in all non-atrophic fibres; activity was low as in type I fibres. Some intermediate fibres were usually present. In 2 cases (Ch 3254 and Ch 2954a) slight differences in myosin ATPase activity were seen from one fascicle to another.

TABLE 2

HISTOCHEMICAL CHARACTERISTICS OF MYOSIN ATPASE TYPE I AND TYPE II GROUPS

No.	*Sex*	*Age (yrs)*	*Duration illness (yrs)*	*Diagnosis*[a]	*Muscle*	*Distribution atrophic fibres*	*Myosin ATPase*	*NADHTR*[b]	*SDH*[b]	*GPOx*[b]	*Phosphorylase*[b]	*Glycogen*[b]
B 9	♂	5½	5	b i s m a	quadriceps	large groups	type I	h	h	l	l	l
B 23	♀	5	4	b i s m a	quadriceps	large groups	type I	h	h	l	l	l
							type II	l	l	h	h	h
B 34	♂	2½	1½	b i s m a	quadriceps	large groups	type I	h	h	l	l	l
							type II	l	l	h	h	h
B 43	♂	35	3	a l s	quadriceps	small groups	type II	l	l	h	h	h
Ch 2480	♂	47	1½	p s m a	triceps brachii	small groups	type II	l	l	h	h	h
Ch 2706	♀	72	2	polyneuropathy (unknown cause)	quadriceps	large groups	type I	h	h	l	l	l
							type II	l	l	h	h	h
H 47	♀	36	1	polyneuropathy (unknown cause)	tib. ant.	disseminated	type I	h	–		l and h	l and h
H 85	♂	44	4	polyneuropathy (unknown cause)	gastrocnemius	small groups	type I	h	–	–	l and h	l and h
H 113	♀	53	1	p s m a	palmaris longus	desseminated	type II	l	–	h	h	–
H 146	♂	39	35	polyneuropathy (unknown cause)	trapezius	disseminated	type I	h	–	l	l and h	l and h
H 150	♀	46	7	p s m a	palmaris longus	large groups	type I	h	–	–	l and h	l and h

[a] see Table 1.
[b] see Table 1.

TABLE 3

HISTOCHEMICAL CHARACTERISTICS OF MYOSIN ATPASE UNIFORM GROUPS

No.	*Sex*	*Age (yrs)*	*Duration illness (yrs)*	*Diagnosis*[a]	*Muscle*	*Distribution atrophic fibres*	*Myosin ATPase*	*NADHTR*[b]	*SDH*[b]	*GPOx*[b]	*Phosphorylase*[b]	*Glycogen*[b]
Ch 2356	♂	21	7	polyneuropathy	tib. ant.	large groups	l	uniform	uniform	l and h	l and h	–
Ch 2441	♂	25	8	b s m a	trapezius	large groups	l	l		l	–	–
Ch 2520	♂	8	4	polyneuropathy	tib. ant.	large groups	l	h	h	l and h	l and h	l and h
Ch 2676	♂	44	20	C–M–T	tib. ant.	large groups	l	uniform	uniform	uniform	uniform	uniform
Ch 2832	♀	53	20	C–M–T	tib. ant.	large groups	l	uniform	uniform	l and h	l and h	l and h
Ch 2954a	♂	60	55	poliomyelitis	tib. ant. (L)	large groups	l	h and l	uniform	l and i	l and i	l and i
Ch 2954b	♂	60	55	poliomyelitis	tib. ant. (R)	large groups	l	uniform	uniform	uniform	uniform	uniform
Ch 3063	♂	18	5–10	C–M–T	tib. ant.	large groups	l	uniform	uniform	uniform	uniform	uniform
Ch 3254	♀	39	35	C–M–T	tib. ant.	large groups	l	i and h	i	l and i	l and h	l and h

[a] see Table 1.
[b] see Table 1.

In 4 cases the parameters of aerobic and glycolytic metabolism showed a uniform pattern, though in general slight variations in activity were seen more often than in the myosin ATPase stain. We did not always feel sure that aerobic metabolism was high in these cases; in fact NADHTR activity was, in our opinion, low at least in 1 case (Ch 2441).

In 5 cases uniformity of myosin ATPase activity was not linked to uniformity of all parameters of aerobic and glycolytic metabolism. In 2 biopsies there was a discrepancy between NADHTR and SDH activity. NADHTR showed groups of slightly varying activity but SDH activity was uniform. Glycolytic metabolism in these 2

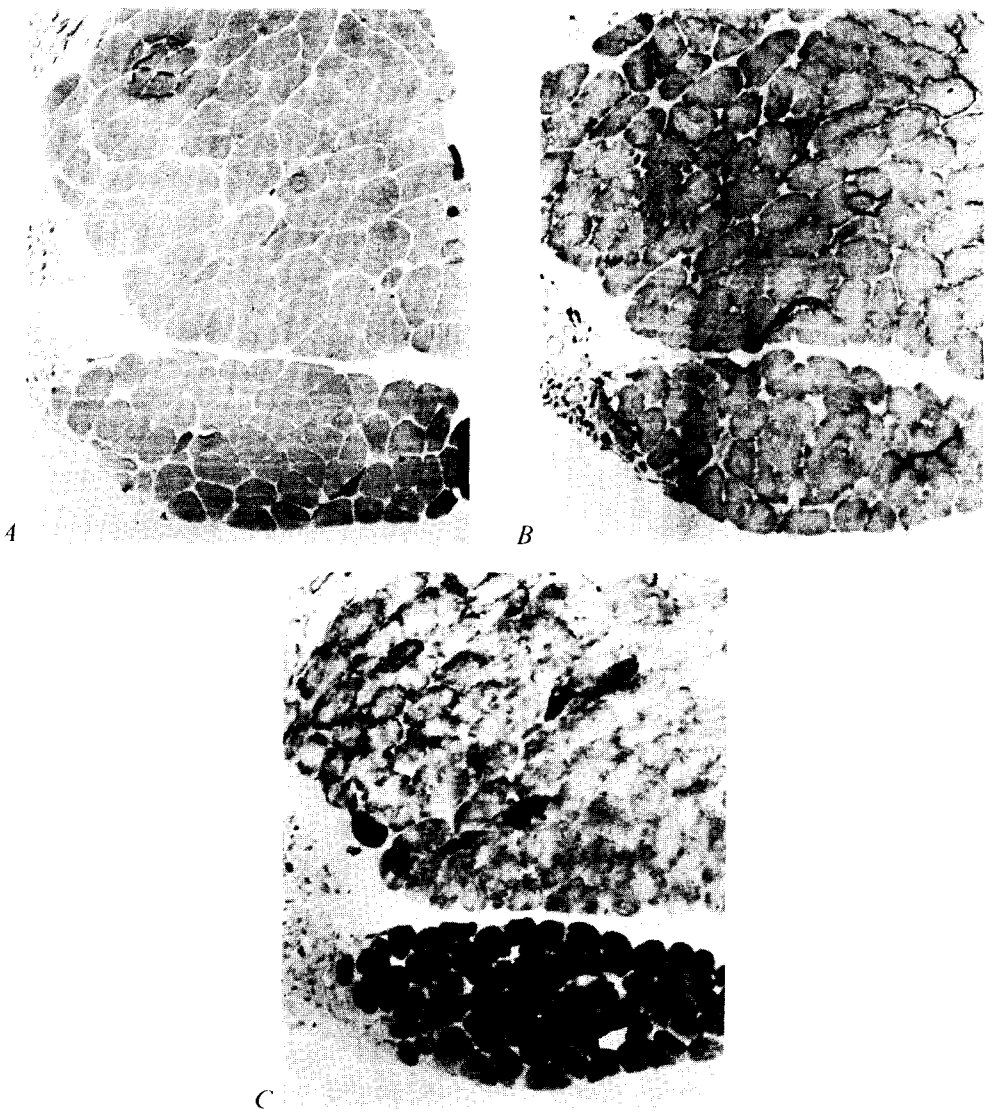

Fig. 3. Case Ch2832, × 120 Serial sections. *A*: myosin ATPase, low activity in fascicles of muscle fibres; *B*: NADHTR, uniform activity; *C*: phosphorylase, activity is high in one fascicle and low in most fibres of the other fascicle.

cases showed 2 fibre types reciprocal to NADHTR activity. In 1 of these 2 cases a group of fibres with low myosin ATPase activity had a low or intermediate level of NADHTR activity. In 3 other biopsies aerobic metabolism was uniform but glycolytic metabolism showed 2 fibre types. In 1 of these 3 biopsies the capacity of glycolytic metabolism showed, in a group-like fashion, marked differences from one fascicle to another (Fig. 3).

B. Biopsies without Type Grouping (Table 4)

According to our criteria type grouping was not present in 15 biopsies, but the mosaic pattern was often abnormal. In 2 cases only a few non-atrophic fibres were preserved; these were disseminated between atrophic fibres and apparently did not belong to one motor unit. They had a low activity of all examined enzymes and a low glycogen content. In most biopsies a number of non-atrophic fibres showed an intermediate level of one or several parameters of the aerobic or glycolytic pathway; intermediate activity was also seen in the myosin ATPase stain.

TABLE 4

BIOPSIES WITHOUT TYPE GROUPING

No.	*Sex*	*Age (yrs)*	*Duration illness (yrs)*	*Diagnosis*[a]	*Muscle*	*Distribution atrophic fibres*
Ch 2276	♂	65	3	a l s	gastrocnemius	most fibres atrophic
Ch 2483	♀	65	$1\frac{1}{2}$	a l s	gastrocnemius	small groups
Ch 2483	♀	65	$1\frac{1}{2}$	a l s	peroneus longus	small groups
Ch 2552	♂	49	$\frac{1}{2}$	a l s	triceps brachii	disseminated
Ch 2570	♂	65	$1\frac{1}{2}$	p s m a	biceps brachii	most fibres atrophic
Ch 2900	♂	62	2	a l s	quadriceps	disseminated
Ch 3368	♂	51	30	C–M–T	quadriceps	disseminated
B 61	♂	5	3	b i s m a	quadriceps	small groups
H 52	♀	66	$\frac{1}{4}$	polyneuropathy (unknown cause)	quadriceps	small groups
H 68	♂	40	$1\frac{1}{2}$	polyneuropathy (unknown cause)	peroneus brevis	disseminated
H 70	♀	22	2	myasthenia gravis	temporalis	disseminated
H 101	♀	62	$\frac{1}{2}$	p s m a	palmaris longus	disseminated
H 122	♂	52	$\frac{1}{2}$	p s m a	palmaris longus	disseminated
H 131	♂	53	$1\frac{1}{2}$	p s m a	quadriceps	disseminated
H 140	♂	63	$2\frac{1}{6}$	p s m a	quadriceps	disseminated

[a] see Table 1.

Investigation of the histochemical characteristics of non-atrophic non-grouped fibres was not within the scope of this study. We were, however, interested to know whether the same fibre hybrids as in type grouping were present in biopsies without the obvious characteristics of reinnervation. Two corresponding fields of strictly serial sections from 3 cases of progressive spinal muscular atrophy were photographed, and enzyme activity and glycogen content were estimated in at least 50 fibres. In 2 cases

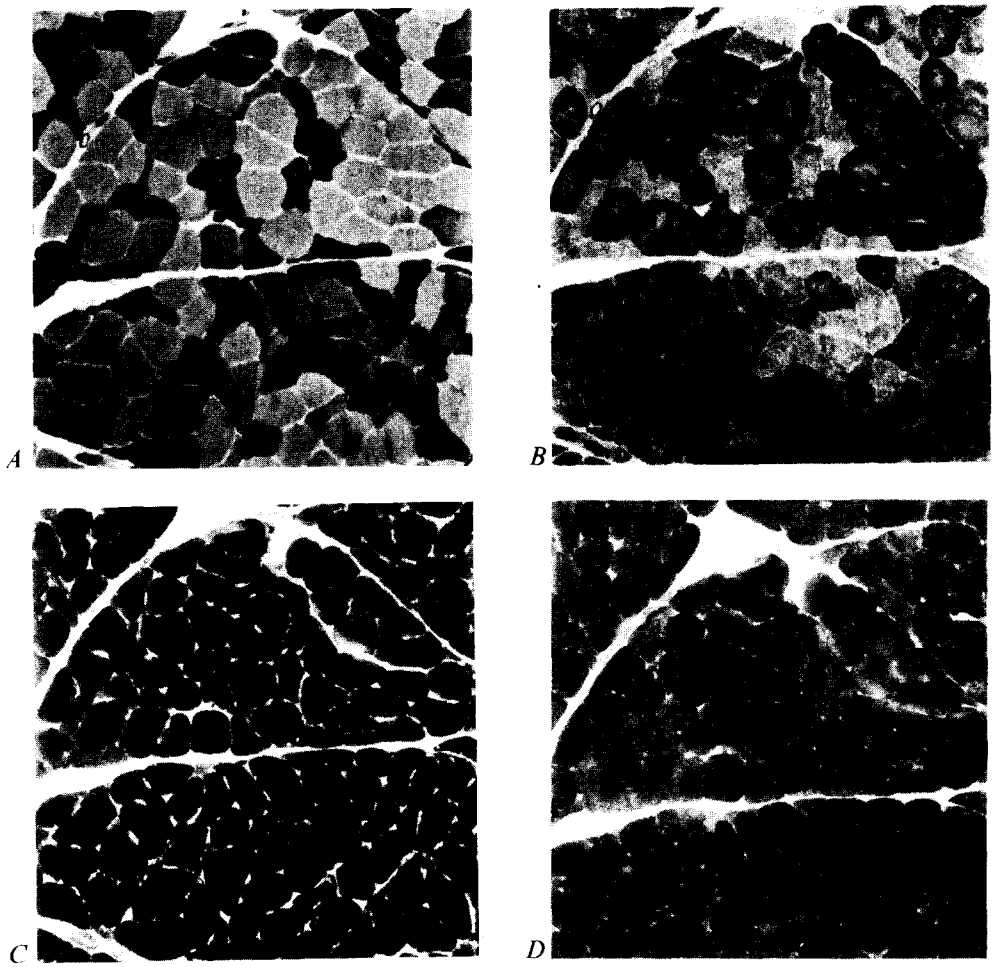

Fig. 4. Case H131, × 100. Serial sections. *A*: myosin ATPase, intermediate activity in 10–15% of muscle fibres; *B*: NADHTR, fibres with intermediate activity often show also intermediate myosin ATPase activity; *C* and *D*: phosphorylase and PAS, few differences in enzyme activity and glycogen content.

denervation atrophy was restricted to a small number of fibres. In these cases aerobic metabolism was high in type I fibres and low or intermediate in type II fibres. Glycolytic metabolism was abnormal in some fibres: in 1 case there was not much difference in phosphorylase activity and glycogen content between fibre types (Fig. 4); the second case showed intermediate or high glycogen content and phosphorylase activity in a minority of type I fibres; few type II fibres had a low glycogen content. Denervation atrophy was well advanced in the third case. Some type I fibres and most type II fibres were atrophic (Fig. 5). Non-atrophic type I fibres had high, intermediate or low NADHTR activity; GPOx activity was intermediate or low, phosphorylase activity and glycogen content on the other hand were high in about 50% of the non-atrophic type I fibres. Few non-atrophic type I fibres had an intermediate or low level of all parameters of metabolism.

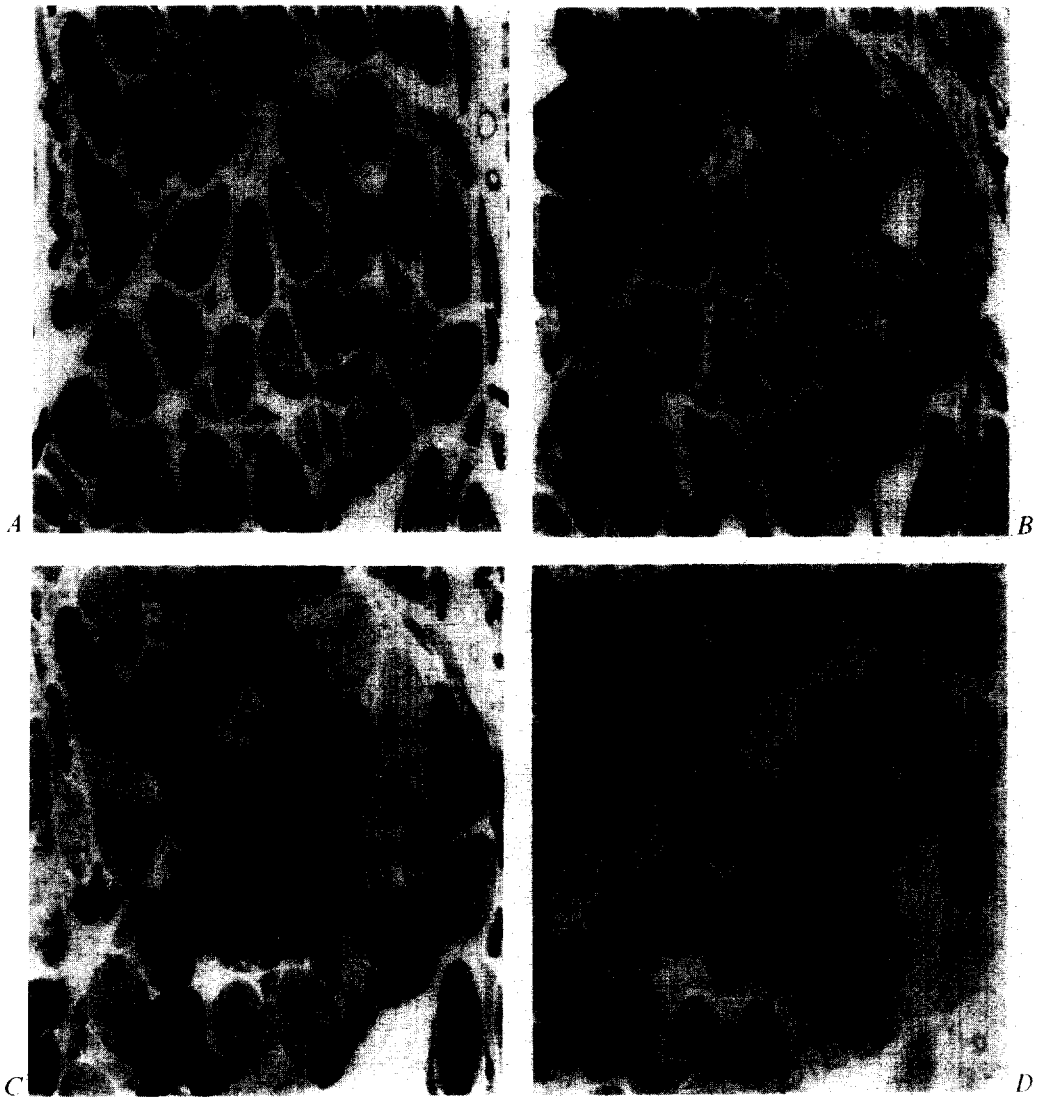

Fig. 5. Case H122. × 160. Serial sections. *A*: myosin ATPase; *B*: NADHTR; *C*: phosphorylase; *D*: PAS. Varying levels of NADHTR and phosphorylase activity and of glycogen content in type I fibres (arrow).

DISCUSSION

Myosin ATPase is probably rate-limiting for the contraction speed of muscle fibres (Close 1972). On the basis of myofibrillar ATPase activity, Engel (1970) recognized 2 fibre types and for human muscle this classification is accepted by most authors. Other authors feel, however, that the results of combined physiological and histochemical research on mammalian skeletal muscle point to 3 distinct fibre types (Close 1972). These authors distinguish "slow twitch fatigue-resisting" fibres, "fast twitch fast-fatiguing" fibres and "fast twitch fatigue-resisting" fibres. Slow twitch fibres have a low myosin ATPase activity and a well-developed aerobic metabolism.

Fast twitch fibres have high myosin ATPase activity and a well-developed glycolytic metabolism, but they differ in their aerobic energy-yielding system: fast-fatiguing fibres have little aerobic metabolism, fatigue-resisting fibres have a high level of aerobic metabolism. The difference between advocates of 2 and those of 3 fibre types is less fundamental than it seems: authors supporting a classification into 2 types accept the need of subdivisions. Baloh and Cancilla (1972) recently compared the myosin ATPase activity with NADHTR and phosphorylase activity in normal human muscle fibres and they decided upon 3 and perhaps 6 combinations. Myosin ATPase type I fibres had a low or intermediate phosphorylase activity and a high NADHTR level. Myosin ATPase type II fibres had a high or intermediate phosphorylase activity: many fibres had a low NADHTR activity but in others it was high.

The main results of the present study can be summarized as follows. Myosin ATPase activity in type groups was often strikingly regular, the tendency to maintain a marked distinction between fibres with high and fibres with low activity being well-preserved. Many myosin ATPase type I groups showed a high capacity of aerobic metabolism and a low capacity of glycolytic metabolism; type II groups were also seen with low or intermediate aerobic and high glycolytic metabolism. Two deviations from this pattern were repeatedly observed. In at least 2 cases myosin ATPase type I groups had an apparently low capacity of aerobic metabolism. In 12 cases fibres or sub-groups of fibres in myosin ATPase type I groups showed an intermediate or high capacity of glycolytic metabolism and this was combined with intermediate or high capacity of aerobic metabolism. Fibres of abnormal type did not only occur in type groups of longstanding neurogenic muscle disease: histochemically abnormal fibres were seen in cases of progressive spinal muscular atrophy without obvious fibre type grouping —a configuration which of course does not preclude reinnervation. The histochemical characteristics of some of these fibres were similar to those seen in type groups.

Fibre hybrids of the type observed in this study are not likely to be specific for neurogenic muscle disease. Several authors have in fact described fibres with both low or both high capacity of oxidative and glycolytic metabolism in Duchenne muscular dystrophy (Dubowitz 1968; Bell and Conen 1970). Baloh and Cancilla (1972) compared parameters of the 2 pathways of metabolism with myosin ATPase activity. Their main finding in Duchenne muscular dystrophy was a shift from glycolytic to aerobic metabolism, but in fact "every conceivable combination of staining reactions was present in the dystrophic muscle". Ringqvist (1973) found intermediate myosin ATPase activity and high NADHTR and phosphorylase activity in 1–45% of all muscle fibres in biopsy material of the masseter muscle from 17 patients with mandibular prognathism. Similar fibres were not seen in the biceps brachialis of the same patients. No myopathic changes, signs of denervation or clinical signs of peripheral nerve lesions were present. The significance of this finding is difficult to evaluate at the present time; Ringqvist thought that the fibres described had either an ontogenetic basis or that they reflected an alteration of muscular function due to abnormal intermaxillary relations.

Fibre hybrids of the type reported in this study have not been noticed in reinnervation experiments. Partly this may be due to the fact that in general either myosin ATPase and parameters of one metabolic pathway or parameters of both pathways

without myosin ATPase were examined. There are, however, reports on incomplete conversion of muscle fibres after reinnervation. Buller and Lewis (1965) observed that reinnervation of cat soleus by fast flexor hallucis longus motoneurones produced no or only very small changes in the force-velocity relation of this muscle: the authors concluded that reinnervation may produce at least one hybrid type of muscle, namely slow muscle reinnervated by phasic (fast) motoneurones. Dubowitz (1968) confirmed the observation of Buller and Lewis: following cross-reinnervation histochemical changes did not develop in cat soleus muscle. He noticed, however, differences between the species. Cross-reinnervation of, *e.g.*, the rabbit soleus did lead to clear-cut histochemical changes. The data available at this moment on incomplete conversion of different parameters of muscle function following experimental reinnervation have recently been summarized (Close 1972).

Few authors have commented upon fibre hybrids in type groups in human neurogenic muscle disease. Mastaglia and Walton (1971) observed fibres with low myosin ATPase activity and low SDH activity in type groups of 5 cases of Kugelberg-Welander disease. Many of these hybrids were hypertrophic or at least normal-sized and it was felt that the low SDH activity could be due to increased exertion and to work hypertrophy. This suggestion was based on Goldspink's observation (1969), in an investigation of mouse muscle at different ages and stages of growth, that SDH concentration was inversely related to fibre growth and myofibrillar content. The influence of change of use, exertion and work overload has since been the subject of many studies. It appears that muscles of primates and of other mammals have the capacity to adapt functionally to hyperactivity by some hypertrophy and by conversion of a number of fast-twitch fast-fatiguing fibres with low aerobic metabolism into fast-twitch fatigue-resisting fibres with high aerobic metabolism (Edgerton, Gerchman and Carrow 1969; Guth and Yellin 1971; Edgerton, Barnard, Peter, Gillespie and Simpson 1972). In some non-physiological conditions changes in activity pattern produced slowing of contraction time, increase of the number of fibres with low (red-like) myosin ATPase activity and increase of fibres with high aerobic and low glycolytic metabolism (Guth and Wells 1972; Romanul, Sreter, Salmons and Gergely 1973). Gutmann, Schiaffino and Hanzliková (1971) studied compensatory hypertrophy of rat soleus muscle fibres. Hypertrophy was induced by functional elimination of synergistic muscle and was shown to be mainly the result of stretch. In early periods of compensatory hypertrophy a decrease of SDH and NADHTR activity developed; late periods were not studied. Decrease of aerobic metabolism in slow-twitch fatigue-resisting fibres following endurance training has not been reported. Clearly, there seems to be a relation between hypertrophy and low aerobic metabolism; it is, however, unlikely that in human reinnervated type I fibres low aerobic metabolism is induced by exertion only.

Dubowitz (1968) observed fibres with intermediate or high aerobic metabolism and high glycolytic metabolism in cases of infantile spinal muscular atrophy. He pointed to the foetal character of this pattern but the weak myosin ATPase activity which was noticed in 1 case was not fully consistent with this suggestion. The presence of these fibre hybrids in adult neurogenic muscle disease shows that arrest of development is an unlikely explanation for their occurrence.

The 2 main kinds of fibre hybrids in our material always had a low myosin ATPase activity. Though it may well be that these fibres have to be considered as incompletely-converted reinnervated type I fibres, it is equally well possible that some of them have originally belonged to type II, *e.g.* in a number of type II fibres myosin ATPase activity may have dropped to the level of type I while the initial level of glycolytic metabolism is preserved. In our material evidence of this kind of abnormal metabolism was present only in a minority of the muscle fibres. The occurrence of these fibres would be explainable if one assumed that they have been reinnervated recently and that conversion of the 2 main pathways of metabolism can develop at a markedly different pace. In our opinion these assumptions are unlikely to explain the existence of whole fascicles of fibre hybrids. Reinnervation in these fascicles has to be conceived as a process that is not achieved for all fibres at the same moment; if conversion develops in a limited time period, a spectrum of varying levels of enzyme activity will manifest itself. As in the muscle fibres of the cat soleus there is, apparently, a lack of plasticity preventing completion of the change to another type, which in turn may be due to an abnormal innervation pattern or to another, as yet unknown, neurogenic factor.

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SUMMARY

Change of fibre type caused by reinnervation implies change in a series of metabolic processes. As long as these changes are in progress the histochemical pattern in muscle fibres may demonstrate deviations from the normal characteristics. The present histochemical study was undertaken to evaluate in human neurogenic muscle disease the completeness of conversion of presumably reinnervated muscle fibres.

At least a number of muscle fibres in type groups is reinnervated. Type grouping of non-atrophic fibres was found in 27 of 42 muscle biopsies from patients with denervating diseases. The myosin ATPase activity in these groups was often strikingly even. In a varying degree and in a varying number of muscle fibres myosin ATPase-uniform groups showed intermediate capacity of aerobic and/or glycolytic metabolism; this finding was considered compatible with conversion due to reinnervation. Two main kinds of fibre hybrids were observed. One kind showed low myosin ATPase activity and an apparently low capacity for aerobic metabolism. The other kind also showed low myosin ATPase activity but the capacity of glycolytic metabolism was high, aerobic metabolism in these fibres being intermediate or high.

It has been suggested that low capacity of aerobic metabolism in fibre hybrids of the first kind is related to hypertrophy of the muscle fibres. The appearance of fibre hybrids of the second kind would be conceivable as a stage in a process of conversion, if at least changes in the capacities of the 2 metabolic pathways can develop at a markedly different pace. However, groups or fascicles of fibre hybrids of this kind are present in some cases. These configurations point to a steady state rather than to a dynamic process of conversion; a lack of plasticity in the muscle fibre apparently prevents completion of conversion.

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