The extensor digitorum brevis: histological and histochemical aspects

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SUMMARY Samples of the extensor digitorum brevis muscle (EDB) obtained at necropsy from 26 subjects without known neuromuscular disease were examined histologically and histochemically. In the two youngest subjects, aged 2 months and 8 years, a mosaic distribution of type I and type II fibres was present. From the second decade onwards, increasing with age, the mosaic pattern was gradually replaced by groups of type I and type II fibres and areas of grouped fibre atrophy appeared. It is suggested that these findings may be explained by a slow process of denervation and reinnervation. This process does not seem to occur to the same extent in three other distal limb muscles from which specimens were also examined.

The extensor digitorum brevis muscle (EDB) is a relatively flat muscle which occupies an exposed position on the dorsum of the foot. Largely on account of its isolation from other muscles and its distal position, this muscle has been used extensively in electromyography with concentric needle electrodes and in measurements of motor nerve conduction velocity. More recently the muscle has been chosen for an electrophysiological method of estimating the number of motor units (McComas, Fawcett, Campbell, and Sica, 1971). These authors discuss at some length the merits and also the disadvantages of this muscle as a preparation for research purposes.

In recent investigations involving the examination of various human skeletal muscles obtained at necropsy (Jennekens, Tomlinson, and Walton, 1971a, b, c) we were struck by the frequent occurrence of appearances suggesting neurogenic atrophy and of groups of type I and type II fibres in the EDB's of subjects who had had no signs or symptoms of neuromuscular disease. As we were unaware of any reports in the literature on the histology and enzyme histochemistry of the EDB, we decided to extend our observations to a larger number of specimens. As biopsy material was not available, necropsy material was used.

Though necropsy material is not suitable for refined enzyme histochemical investigations, it

has been shown (Susheela and Walton, 1969; Jennekens *et al.*, 1971a, b) that type I and type II fibres can readily be distinguished in striated muscles taken at necropsy within 48 hours of death. This allows observations to be made on the numbers, sizes, and shapes of the two fibre types and on their distributions in relatively large transverse sections of several muscles in one individual. In the present study the EDB was compared with the flexor digitorum brevis (FDB), the tibialis anterior (TA), and the gastrocnemius (G) muscles.

TA and EDB are innervated mainly by nerve fibres running through the anterior root of L5 and S1, the G and FDB are innervated mainly by nerve fibres from S1 and S2 spinal nerves. The posterior tibial nerve is the peripheral nerve trunk innervating G and FDB, the TA and EDB are supplied by branches of the common peroneal nerve. EDB and FDB lie at approximately the same distance from the anterior horn cells in the spinal cord and are therefore innervated by motor neurones with axons of approximately equal lengths.

METHODS

Sections of the EDB were obtained from 26 patients (Table). None of the patients had suffered before death from any disease which was either long-lasting or prostrating. There was no evidence of previous neuromuscular disease in any patient and most had died as a result of trauma or an acute illness.

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TABLE
'FIBRE TYPE GROUPING' IN FOUR DISTAL LIMB MUSCLES IN NECROPSY MATERIAL FROM 26 SUBJECTS

Case	Age (yr)	Sex	Clinical data	Principal necropsy finding		Grade of grouping (see text)			
						G	TA	FDB	EDB
1	1/2	M	Progressive cardiac failure	Congenital heart anomaly	Rt	0	0	0	0
					Lt	-	-	-	0
2	8	M	Sudden death	Head injury		0	0	0	1
3	11	M	Sudden death	Head injury		0	0	0	2
4	18	M	Sudden death	Head injury		0	0	2	2
5	18	M	Sudden death	Head injury		-	0	0	2
6	19	F	Epilepsy, sudden death	Head injury		-	-	-	2
7	27	M	Sudden death	Barbiturate overdose		-	-	-	2
8	33	M	Sudden death	Head injury		-	-	-	3
9	33	F	Sudden death	Head injury		0	1	1	2
10	35	F	Sudden death	Barbiturate overdose		0	-	1	3
11	35	F	Sudden death	Myocardial infarction		-	-	-	3
12	42	F	Sudden death	Head injury		-	-	-	3
13	45	M	Sudden death	Myocardial infarction		0	1	1	3
14	60	M	Influenza, succumbed within 7 days	Bronchopneumonia		1	1	-	3
15	63	M	Influenza, succumbed within 7 days	Bronchopneumonia		0	0	1	3
16	65	F	Succumbed in 6 days	Myocardial infarction		-	-	-	3
17	67	M	Sudden death	Fractures of cervical vertebrae		-	-	-	3
18	69	F	Spells of dizziness, sudden death	Head injury	Rt	0	-	1	2
					Lt	1	-	0	3
19	69	M	Influenza, succumbed in 4 days	Bronchopneumonia		-	2	3	3
20	73	F	Gastrointestinal haemorrhage	Gastric ulcer, hiatus hernia		1	0	-	2
21	78	M	Succumbed in 4 days	Myocardial infarction		-	-	-	3
22	80	F	Succumbed in 14 days	Myocardial infarction	Rt	-	-	-	2
					Lt	-	-	-	3
23	80	F	Sudden death	Cerebral haemorrhage		1	2	2	3
24	84	M	Congestive cardiac failure, bronchitis	Myocardial fibrosis		-	-	-	3
25	85	M	Right hemiplegia, sudden death	Cardiac tamponade	Rt	-	-	-	3
					Lt	-	-	-	3
26	88	F	Giddiness, confusion, sudden death	Myocardial infarction		-	-	-	3

-: not analysed. 0: no grouping.

In cases 1, 18, 22, and 25 specimens were taken from both sides of the body; in all other cases material was collected from one side only. In 14 of the 26 patients specimens were also taken from the G, TA, and FDB.

A part of each specimen was frozen in hexane which had been cooled either in liquid nitrogen or in a slush of ice of carbon dioxide and alcohol. Transverse sections of 10 μ thickness and up to 2.5 cm² were cut in a cryostat at a temperature of -20°C. The activity of myofibrillar ATP-ase and of diaphorase (NADH diaphorase) was determined according to the methods of Padykula and Herman (1955) and of Scarpelli, Hess, and Pearse (1958) respectively. Other transverse sections were stained with haematoxylin and eosin. Material which had been fixed in 10% formalin and which had been embedded in paraffin was sectioned transversely as well as longitudinally and stained with haematoxylin and eosin and phosphotungstic acid haematoxylin.

In this material a gradual transition with increasing age was observed from a pure mosaic pattern on the one hand to complete grouping with large groups in a transverse section of the whole muscle on the other hand. To facilitate comparison, the extent of

grouping was assessed semiquantitatively. If a transverse section was composed largely of a mosaic pattern with groups of at least 60 fibres in small parts of the section, grouping was classified as being grade 1. Grouping was classified as grade 2 if it occurred in about 50% of the whole section and as grade 3 if groups occupied the whole or nearly the whole of the transverse section.

RESULTS

The volume of the four limb muscles examined was often somewhat less in old age than in youth. This decrease was particularly striking in the EDB. In young people the belly of this muscle causes the skin of the lateral half of the dorsum of the foot to bulge upwards slightly above the forepart of the calcaneus and above the cuboid bone. At necropsy this muscle appeared atrophic in patients between 60 and 90 years of age when compared with its appearance in subjects younger than 20 years. The EDB of seven of the 16 specimens examined from patients between 60 and 90 years old had largely been replaced by connective tissue and fat.

FIG. 1. Transverse cryostat section from the EDB of a baby of 2 months. Myosin ATP-ase, $\times 120$. Distribution of type I and type II fibres in a mosaic pattern.

HISTOLOGY AND HISTOCHEMISTRY OF EDB There was a fine mosaic pattern in the transverse sections of this muscle in a baby of 2 months (Fig. 1) and in a boy of 8 years. In this latter case a group of about 100 type I fibres was seen at one side of the section, corresponding to the innermost part of the muscle. This innermost part showed a slight preponderance of type I fibres; in the outer, more dorsal part there was a slight preponderance of type II fibres.

The EDB of case 3, a boy of 11 years, presented an entirely different picture. Small groups of 30 to 80 histochemically uniform fibres (Fig. 2) were scattered all over the sections, leaving a mosaic pattern in less than 50% of the transverse sec-

tions. Though groups of type I fibres occurred slightly more frequently, groups of type II fibres were present as well. There was no obvious increase in the variation of fibre size, either in different groups or in different fields of the sections.

With minor variations, the same picture as that seen in case 3 appeared to be present in the EDB of all other cases up to the age of 30 years (cases 4, 5, 6, and 7). Amid the mosaic pattern groups of both fibre types were spread all over the sections. In case 4 a group of 20 to 30 atrophic fibres was lying at the extreme edge of the cryostat section. We were not sure how to interpret this finding. In case 7 the variation in fibre size was

FIG. 2. Transverse cryostat section from the EDB of a boy of 11 years. Myosin ATP-ase, $\times 120$. Small groups of type I and type II fibres.

FIG. 3. Transverse cryostat section from the EDB of a man of 27 years. Myosin ATPase, $\times 120$. Atrophic and elongated fibres between others of normal size.

thought to be beyond the normal range. Isolated, atrophic, angulated fibres (Fig. 3) were seen among others of normal size.

In patients between 30 and 50 years of age, groups generally tended to be of a larger size than in patients below the age of 30 (Fig. 4). Some evidence of a mosaic pattern remained in the EDB of case 9, but groups only were seen in cases 8, 10, 11, 12, and 13. The variation in fibre size was within the normal range in three specimens, but abnormal in three others. In case 13 fibres within groups were often surprisingly uniform in size, but there was an abnormal variation in fibre size between different groups. A small bundle of atrophic fibres of type I was seen in case 10 (Fig. 5) and many bundles of

atrophic fibres occurred in case 11 (Fig. 6); the characteristic appearances of neurogenic atrophy were thus present in both of these cases.

It was not so much the extent of type grouping as the frequency of grouped fibre atrophy which marked the difference between patients of 30 to 50 years and those of 60 to 90 years. Only three of 13 patients in the 60 to 90 year category showed a recognizable mosaic-like pattern in some small parts of the EDB; in all other patients in this age group the sections of the EDB were completely occupied by groups which were often large. Groups of type I fibres were slightly more frequent than groups of type II fibres. Groups of intermediate fibres were seen frequently in sections stained for NADH diaphorase activity.

FIG. 4. Transverse cryostat section from the EDB of a man of 45 years. DPNH diaphorase, $\times 50$. Fibre type grouping, the mosaic pattern is almost completely absent.

FIG. 5. Transverse cryostat section from the EDB of a woman of 35 years. Myosin ATP-ase, $\times 120$. Grouping of type I and type II fibres and a group of atrophic type I fibres.

In case 17, all of the fibres which were not atrophic were histochemically uniform.

Evidence suggesting denervation atrophy was present in the EDB of 11 out of 13 elderly (60 to 90 years) patients. In four specimens only a few fascicles were not atrophic. Type I, intermediate, and type II fibres in three of these specimens were large or hypertrophic, the shortest diameters (Jennekens *et al.*, 1971a) of the largest fibres being $100\ \mu$ in case 17, $130\ \mu$ in the left EDB of case 25, and $180\ \mu$ in case 21 (Fig. 7). These large fibres had acquired a rounded shape and some contained internal nuclei (Fig. 8). Occasional fibres in specimens of this age group showed a target-like appearance, irregular distribution of oxidative enzyme activity in type

I fibres and fibre necrosis. It is, of course, possible that such irregularity of oxidative enzyme activity could be due to postmortem autolysis (Jennekens *et al.*, 1971c).

HISTOLOGY AND HISTOCHEMISTRY IN THE FDB, TA AND G Material was removed from these three muscles in eight subjects varying in age from months to 45 years. Histological examination did not reveal any obvious pathological changes. Grouping of type I and of type II fibres occurred in four of eight specimens of the FDB and was classified as either grade 1 or grade 2. Grouping was infrequent in the TA and G and was never greater than grade 1.

Grouping and signs of neurogenic atrophy

FIG. 6. Transverse cryostat section from the EDB of a woman of 35 years. Myosin ATP-ase, $\times 120$. Fibre type grouping and a group of atrophic fibres; some intermediate fibres are present.

FIG. 7. Transverse cryostat section from the EDB of a man of 78 years. DPNH diaphorase, $\times 120$. Fibre hypertrophy, irregular distribution of oxidative enzyme activity in some fibres.

were, however, seen regularly in specimens of the same muscles obtained from patients between 60 and 90 years of age. The FDB was completely grouped in one case; in all other specimens the mosaic pattern was preserved, at least partly. In FDB and G both groups of type I and of type II fibres were present; in the TA grouped fields were usually composed of type I fibres; occasionally, however, small groups of type II fibres occurred. Grouped fibre atrophy was seen in three of six specimens of the FDB. Even when evidence of neurogenic atrophy was widespread, many fascicles were spared. Fibre hypertrophy was not observed. Signs of neurogenic atrophy were seen in four specimens of the G and in one specimen of the TA.

Grouping could not be graded in the G of case 19, due to extensive necrobiotic changes which were probably caused by ischaemia; the TA, FDB, and EDB of this patient were not similarly affected. Fibres with a target-like or targetoid-like appearance were seen in several specimens (Fig. 9). Irregular distribution of oxidative enzyme activity in type I fibres, fibre necrosis, and phagocytosis were also observed in occasional fibres of several specimens (Jennekens *et al.*, 1971c).

Statistical analysis of the extent to which grouping occurred showed that the four limb muscles examined were not identical in this respect (Friedman's test, $P < 0.01$). There was significantly more grouping in the EDB than in

FIG. 8. Transverse cryostat section from the EDB of a man of 67 years. Haematoxylin and eosin, $\times 120$. Groups of atrophic fibres. Rounded appearance of some fibres and fibres with internal nuclei.

FIG. 9. Serial transverse sections from the gastrocnemius muscle of a woman of 80 years (case 23) $\times 480$. A target-like fibre. (a) DPNH diaphorase, a zone of low enzyme activity in a type I muscle fibre; (b) myosin ATP-ase, there is a zone of low enzyme activity surrounded by a ring of slightly higher activity in the same fibre; (c) haematoxylin and eosin, at the site of low enzyme activity there is a purple core. Another muscle fibre (bottom left) shows a small zone of similar characteristics.

the FDB (Wilcoxon's matched-pairs signed ranks test, $P < 0.01$), in the TA (ditto, $P < 0.01$), and in the G (ditto, $P < 0.01$). The figures suggested that grouping occurred non-significantly more often and more extensively in the FDB than in the TA and in the G. No difference could be shown between the TA and the G.

DISCUSSION

Recently it has been said that muscle fibres of one motor unit are intermingled, within a territory of some millimetres, with fibres of other motor units (Ekstedt, 1964; Edström and Kugelberg, 1968; Mayer and Doyle, 1969). This finding is pertinent to the interpretation of the phenomenon of grouped fibre atrophy in neurogenic diseases. Interruption of a motor axon results in denervation of a number of isolated muscle fibres. It is now known that these fibres may be reinnervated by sprouts of nearby motor axons of other motor units. Volumetric changes in denervated muscle fibres may be prevented when sprouting occurs rapidly (Hildebrand, Joffroy, and Coërs, 1968). Enlargement of motor units by collateral ramification and reinnervation increases the likelihood that muscle fibres of one unit will be grouped into sub-units. Assuming

that the muscle fibres of a motor unit are histochemically uniform (Edström and Kugelberg, 1968; Mayer and Doyle, 1969), reinnervation then results in grouping of fibres of one type. It is likely, too, that following recent reinnervation, muscle fibres may show an intermediate intensity of enzyme staining while in the process of changing from one fibre type into another; thus a finding of many intermediate fibres could suggest recent reinnervation. The degree of fibre type grouping is probably related to the amount of collateral ramification (Karpati and Engel, 1968). Subsequent denervation of such previously enlarged motor units will then lead to grouped fibre atrophy which will probably be even more striking if axonal sprouting has been inadequate. Muscle fibre atrophy in neurogenic disease is the result of denervation combined with insufficient reinnervation (Coërs, 1969).

In the EDB fibre type grouping occurred strikingly often, sometimes in young adults. We believe that this grouping is probably caused by a process of denervation and reinnervation. A grouped pattern was not apparently present in early infancy, but it subsequently developed gradually, as was shown by the mosaic pattern in the two youngest children and by the change from small groups with some residual evidence

of a mosaic pattern in the age range between 10 to 30 years, to large groups in older individuals. An alternative explanation would be to suggest that in man, as occurs in some muscles of the lower mammals, other causes for grouping of type I and type II fibres than denervation and subsequent reinnervation are conceivable. However, the concept of a continuing process of partial denervation and reinnervation occurring in adult life seems to us more likely. Thus we found few if any groups of atrophic fibres of uniform histochemical type in children and young adults. On the other hand, in middle age and in elderly subjects groups of atrophic fibres of one histochemical type, showing the characteristics of neurogenic atrophy, were seen with increasing frequency; this finding suggested that there had probably been a previous redistribution of fibres of one motor unit into sub-units.

Effective sprouting depends on the survival of an adequate number of motor axons. When the number of motor axons which are available for collateral reinnervation has decreased, the chance increases that the distance between a denervated muscle fibre—or a group of denervated muscle fibres—and the nearest axon will not be bridged (Weiss and Edds, 1946). This explains the increase in grouped fibre atrophy in older patients. In the EDB's of some of the oldest patients even 'myopathic' changes and fibre hypertrophy occurred, exactly as may be seen in motor neurone disease and in chronic neuropathies.

There was less grouping and denervation in the three other limb muscles. If more material had been examined it might have been possible to demonstrate a difference between the FDB and the TA and G, but even in the FDB grouping occurred significantly less often than in the EDB.

There is no evidence to indicate a particular liability of the nerves which innervate the EDB to traumatic lesions. The position of these nerve fibres in the common peroneal nerve is not particularly exposed (Sunderland, 1968). It is, however, known that the anterior tibial nerve shows substantial variation in fibre density (Gairns, Garven, and Smith, 1960; Swallow, 1966) and a decrease of fibre density with increasing age. Gairns *et al.* (1960) suggested badly fitting shoes as a possible cause for the occurrence of low fibre densities in the digital nerves of young subjects. The motor nerves of the EDB at their entrance into this muscle might also be compressed intermittently by shoes. This hypothesis would be supported if non-shoe-wearing indi-

viduals were found to retain a mosaic pattern of fibre types into late life. Further, we were often surprised at postmortem examination to find how firmly the EDB was compressed by the tautly stretched tendons of the extensor digitorum longus muscle on their way to the digits. Entrapment caused by one or both of these factors might explain the occurrence of changes suggesting denervation at a relatively early age, the exceptional regularity with which type groups of muscle fibres occur, and the slowness of the process of denervation and reinnervation in the EDB.

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