

Intercostal muscle biopsy in human neuromuscular disease

Histochemical and electron microscopic studies¹

LAWRENCE Z. STERN², CLAIRE M. PAYNE, RAPHAEL GRUENER, ROBERT M. ANDERSON, AND LINDA K. HANNAPEL

From the Departments of Neurology, Pathology, Physiology and Surgery, The University of Arizona College of Medicine, Tucson, Arizona, U.S.A.

SYNOPSIS External intercostal muscle biopsies were examined histochemically and by electron microscopy. The use of this muscle allowed correlation with physiological and pharmacological studies on the same specimens. Changes observed in muscular dystrophy and motor neurone disease resembled those previously described in biopsied limb muscle and underline the particular usefulness of this preparation in the study of human neuromuscular disease.

The external intercostal muscle preparation has been shown to be a valuable one in various studies of normal and diseased human muscle (Creese *et al.*, 1957; Elmqvist *et al.*, 1960; Elmqvist *et al.*, 1964; Elmqvist and Quastel, 1965; Ludin, 1969, 1970; Santa *et al.*, 1972a, b; Hofmann *et al.*, 1973; Stern *et al.*, 1974; Gruener *et al.*, 1975). Because the biopsy specimen provides intact fibres, it allows correlation of morphological, physiological, and pharmacological results from the same specimen.

Histochemical profiles of a number of human limb muscles are available (Brooke and Engel, 1969; Edström and Nyström, 1969; Jennekens *et al.*, 1971a, b). The purposes of this study were to provide a profile of external intercostal muscle and to document that the morphological changes which occur in this muscle are similar to those found in biopsied limb muscle from other patients with neuromuscular disorders.

METHODS

Intercostal muscle biopsies were performed on four

¹ Some of these data were presented at the IIIrd International Congress on Muscle Diseases, Newcastle upon Tyne, September 1974. Supported in part by grants from the Muscular Dystrophy Associations of America, Inc., N.I.H. grant NS 10417 and a gift from Mr and Mrs Edward Watz.

² Address for correspondence: Dr Lawrence Z. Stern, Department of Neurology, The University of Arizona, Arizona Medical Center, Tucson, AZ 85724, U.S.A.

(Accepted 22 April 1975.)

patients with Duchenne type muscular dystrophy, five with limb-girdle dystrophy, 10 with myotonic dystrophy, three with motor neurone disease, and eight adults under the age of 60 years without evidence of neuromuscular disease. Specimens of external intercostal muscle were removed from the sixth intercostal space in the midaxillary line under posterior intercostal nerve block anaesthesia. A rim of periosteum was left attached to either end of all specimens to ensure that intact muscle fibres were obtained. Immediately upon removal, specimens were placed in a continuously oxygenated cold physiological saline solution (Gruener *et al.*, 1975) and taken to the laboratory. Specimens were dissected clean of adipose and connective tissue and divided into portions used for morphological and physiological studies. The results of the latter studies will be reported separately (Gruener *et al.*, in preparation).

The portion of each biopsy specimen for histochemistry was quenched in isopentane cooled with liquid nitrogen and cross-sectioned at 10 μ m thickness in a cryostat. Sections were stained with the modified Gomori trichrome (Engel and Cunningham, 1963) and the nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) reaction (Farber *et al.*, 1956). Muscle fibre typing was done using the pH 9.4 myofibrillar adenosine triphosphatase (ATPase) reaction (Padykula and Herman, 1955). Histochemical subtyping of the type II fibres was accomplished using the acid preincubation techniques described by Brooke and Kaiser (1970).

Muscle fibre diameters were determined from photographs of representative transverse sections. At least 100 randomly selected fibres were measured from each specimen. Diameters were determined using the greatest distance between opposite sides across the narrowest aspect of each fibre. Megahistogram means were determined according to the method described by Brooke and Engel (1969).

Specimens for electron microscopy were fixed in phosphate-buffered 3% glutaraldehyde, post-fixed in phosphate-buffered 1% osmium tetroxide, and embedded in Spurr's epoxy resin (Spurr, 1969). Thick sections (0.5 μm) were cut and stained with toluidine blue and examined with the light microscope. Thin sections were mounted on uncoated copper grids and stained with uranyl acetate and lead citrate. The grids were then lightly carbon-coated and examined with the Hitachi HU-12 electron microscope.

TABLE
INTERCOSTAL MUSCLE FIBRE DIAMETERS* AND TYPE PERCENTAGES†

Diagnosis, sex	Cases (no.)	Fibre type I	Fibre type II	% type I
Normal				
Male	4	52.4 \pm 0.7	53.6 \pm 0.6	50
Female	4	45.5 \pm 0.6	46.3 \pm 0.9	63
Duchenne				
Male	4	37.1 \pm 0.4	32.2 \pm 0.6	64
Limb-girdle				
Male	3	56.1 \pm 1.4	70.4 \pm 1.2	74
Female	2	58.4 \pm 1.9	56.4 \pm 5.5	98
Myotonic				
Male	6	47.9 \pm 0.7	57.1 \pm 0.6	58
Myotonic				
Female	4	44.6 \pm 0.9	57.5 \pm 1.2	58
Motor neurone				
Male	3	66.6 \pm 1.9	62.4 \pm 2.4	65

* Values are megahistogram means in $\mu\text{m} \pm \text{SEM}$. P values, determined using Student's *t* test, are stated in text.

† More than 100 randomly selected fibres were analysed in each biopsy.

RESULTS

HISTOCHEMISTRY (Figs 1 and 2) Intercostal muscle fibre diameters and type percentages are summarized in the Table. The distribution of fibre types showed a mosaic pattern similar to that of other human skeletal muscles. There was no significant ($P > 0.05$) difference between the mean diameters of type I and type II fibres in either males or females. Both fibre types were, however, significantly ($P < 0.001$) larger in the men than in the women. No other differences were noted comparing normal male and female

muscles, except for a higher percentage of type I fibres in the females (Table and Fig. 1). Each biopsy from the diseased group showed changes previously described as characteristic of that condition in biopsied limb muscle (for example, Dubowitz and Brooke, 1973), though generally less severe.

Biopsy specimens from patients with Duchenne muscular dystrophy showed varying degrees of degeneration and regeneration, variation in fibre size, proliferation of endomysial and perimysial connective tissue, and cellular reactions (Fig. 2A). The findings were quite similar in limb-girdle dystrophy, but with a striking additional

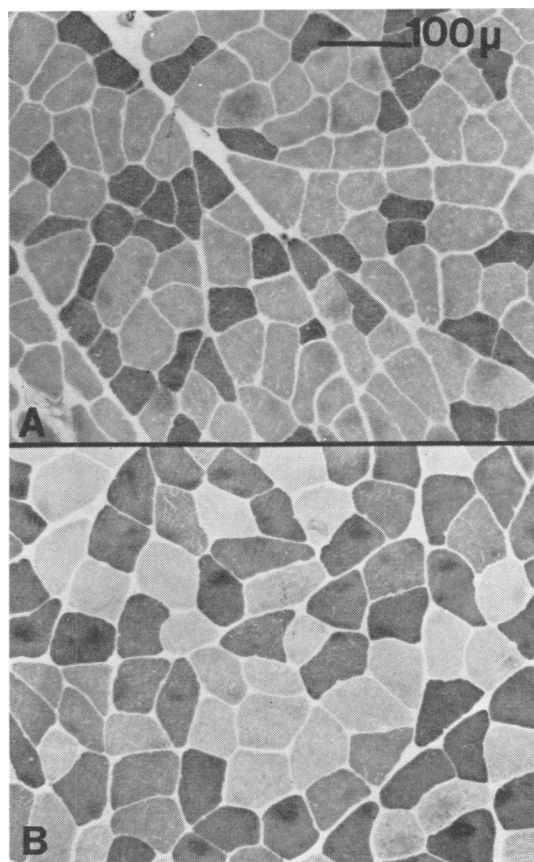


FIG. 1 Normal external intercostal muscle. A. Biopsy specimen from a 33 year old female. B. From a 35 year old male. Note the mosaic pattern and that both type I and type II fibres are larger in the male. pH 9.4 ATPase reaction.

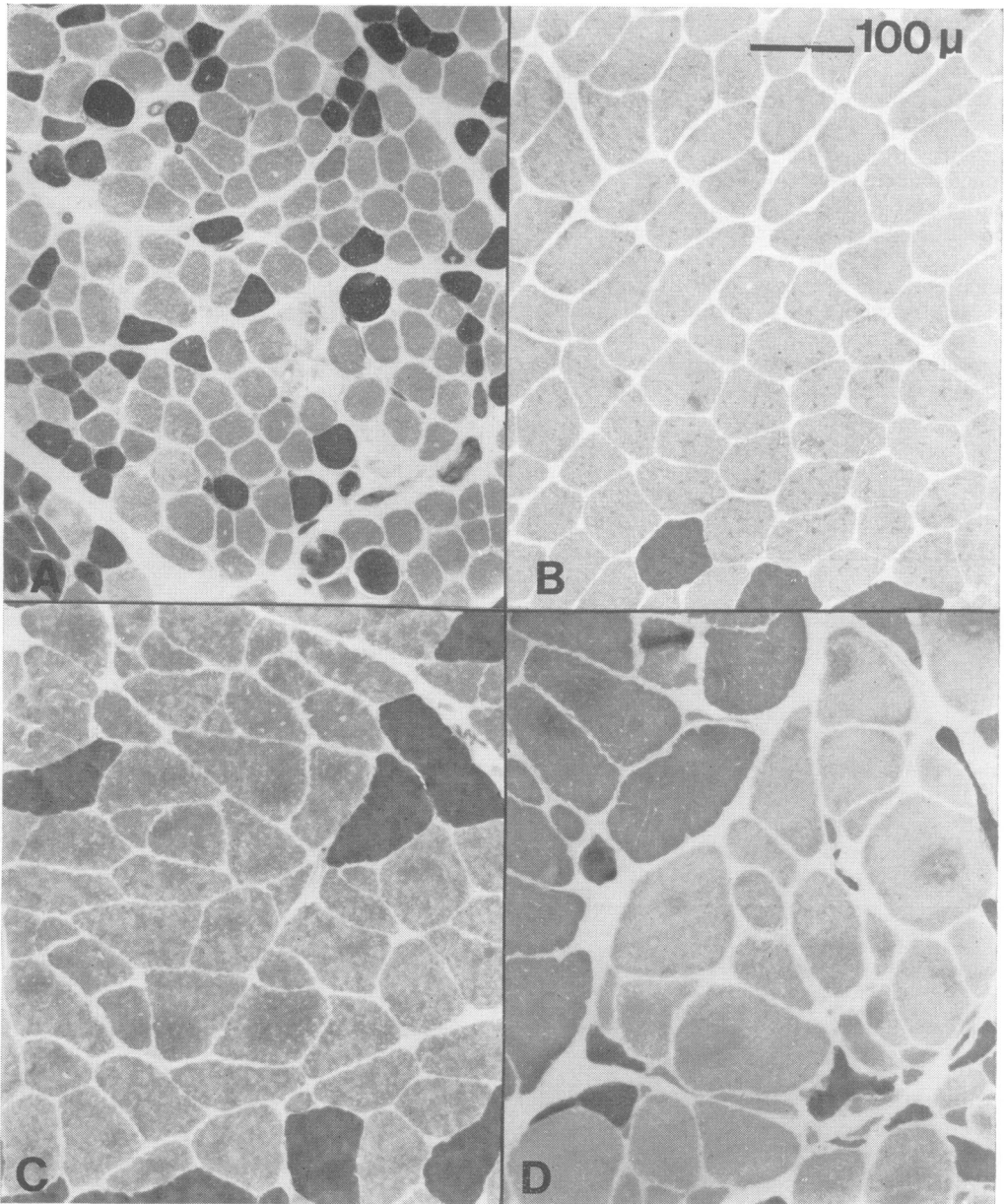


FIG. 2 *Diseased intercostal muscle. A. From a 9 year old male with Duchenne muscular dystrophy showing evidence of degeneration and regeneration, variation in fibre size and some connective tissue proliferation. B. From a 13 year old female with limb-girdle dystrophy showing marked type I fibre predominance. C. From a 45 year old male showing atrophy of some type I fibres. D. From a 50 year old male with motor neurone disease showing small angular fibres, atrophy and hypertrophy of both fibre types, grouping of atrophic fibres and type grouping. pH 9.4 ATPase reaction.*

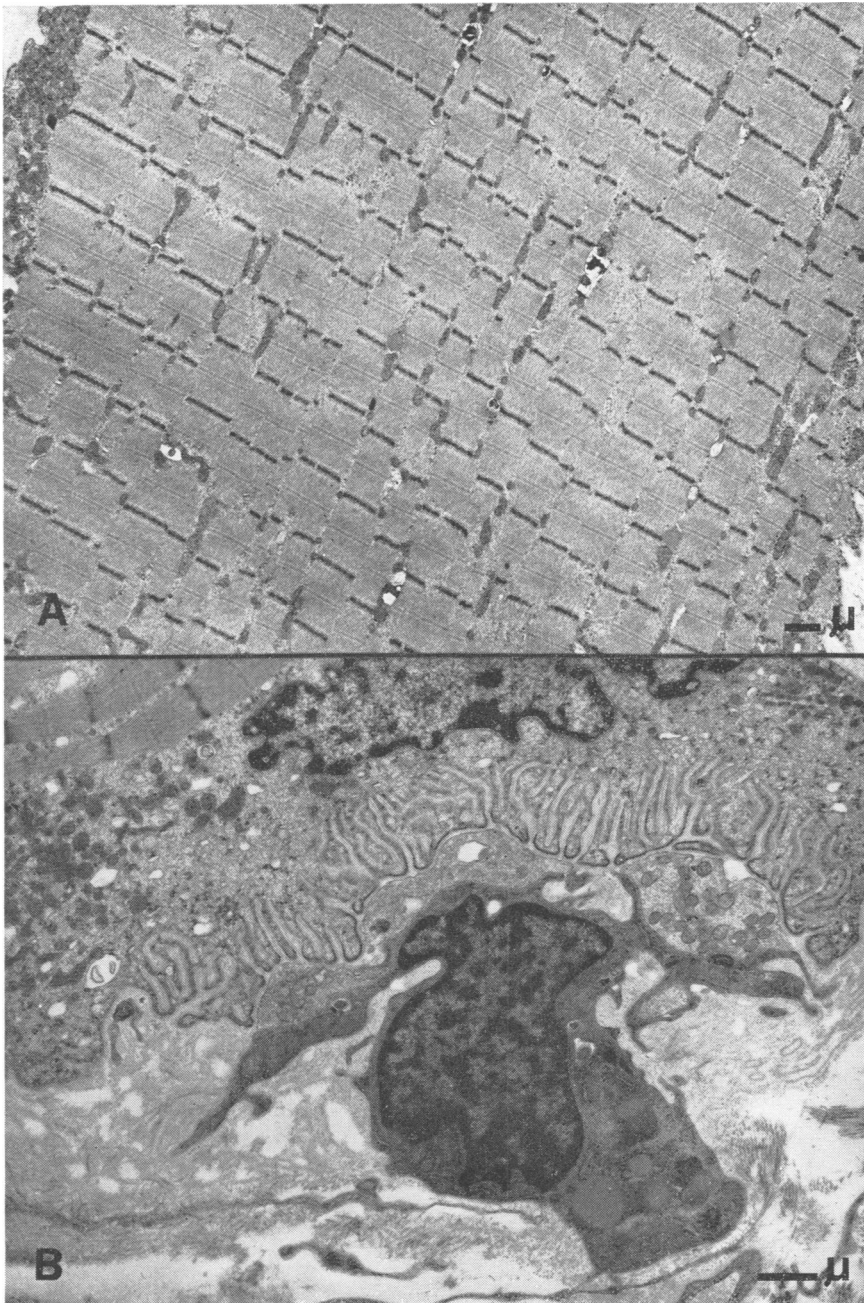


FIG. 3 Normal external intercostal muscle. A. From a 25 year old female showing a normal myofibrillar pattern, scattered mitochondria and lipid droplets. A small mitochondrial aggregate is present at upper left. Myofibrils are clearly delineated. A small amount of glycogen is present, mainly in intermyofibrillar locations. B. From a 33 year old female showing a normal motor endplate. The primary clefts of the subneural apparatus are easily distinguished and the secondary synaptic clefts are roughly parallel to each other.

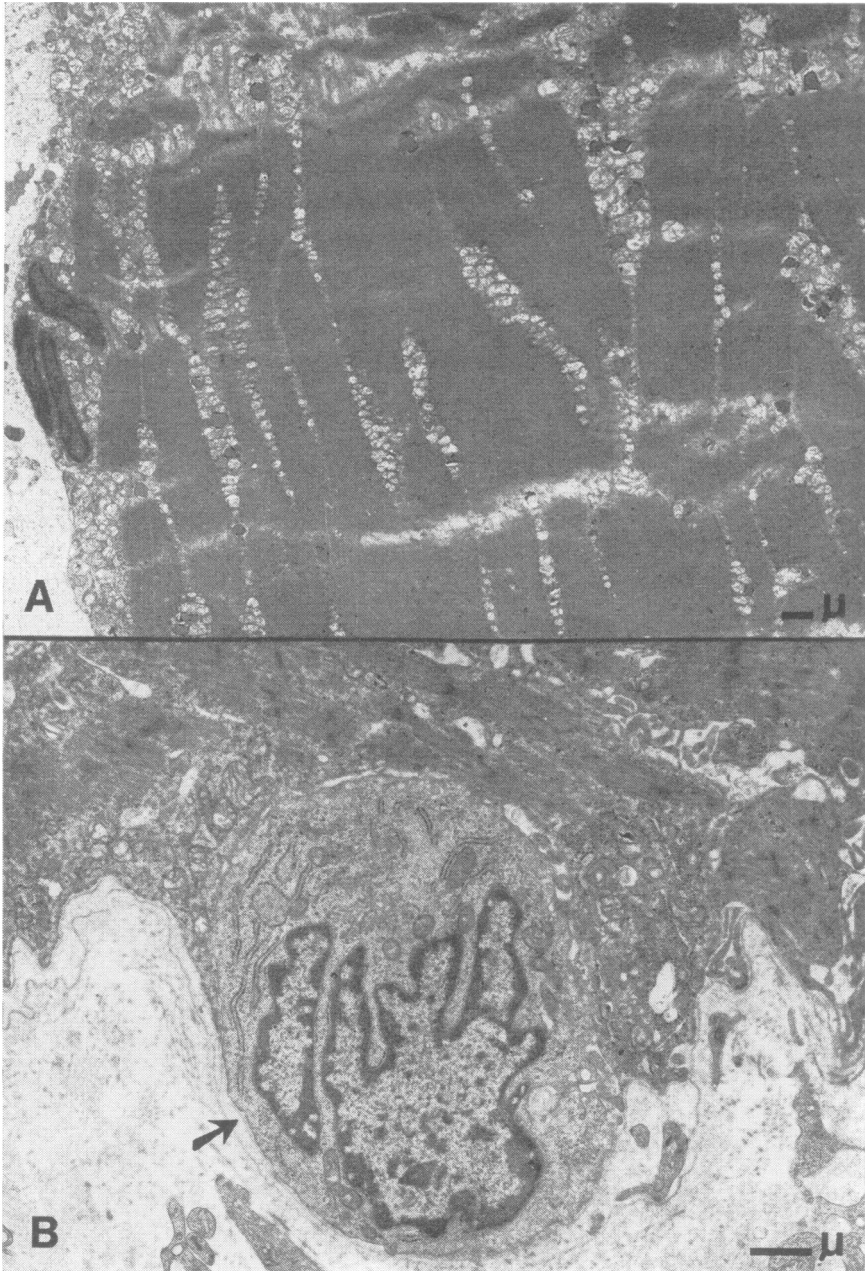


FIG. 4 *Duchenne muscular dystrophy. A. From a 9 year old male showing an advanced stage of hyaline degeneration in which the fibrils are congealed in an extremely electron-dense mass. The mitochondria appear swollen and three sarcolemmal nuclei are clumped in this necrotic fibre. B. From a 7 year old male, showing redundancy of the sarcolemmal basal lamina of an atrophic muscle fibre. The outer surface of the satellite cell is covered by the sarcolemmal basal lamina (arrow). The cytoplasm of the satellite cell contains many profiles of rough endoplasmic reticulum.*

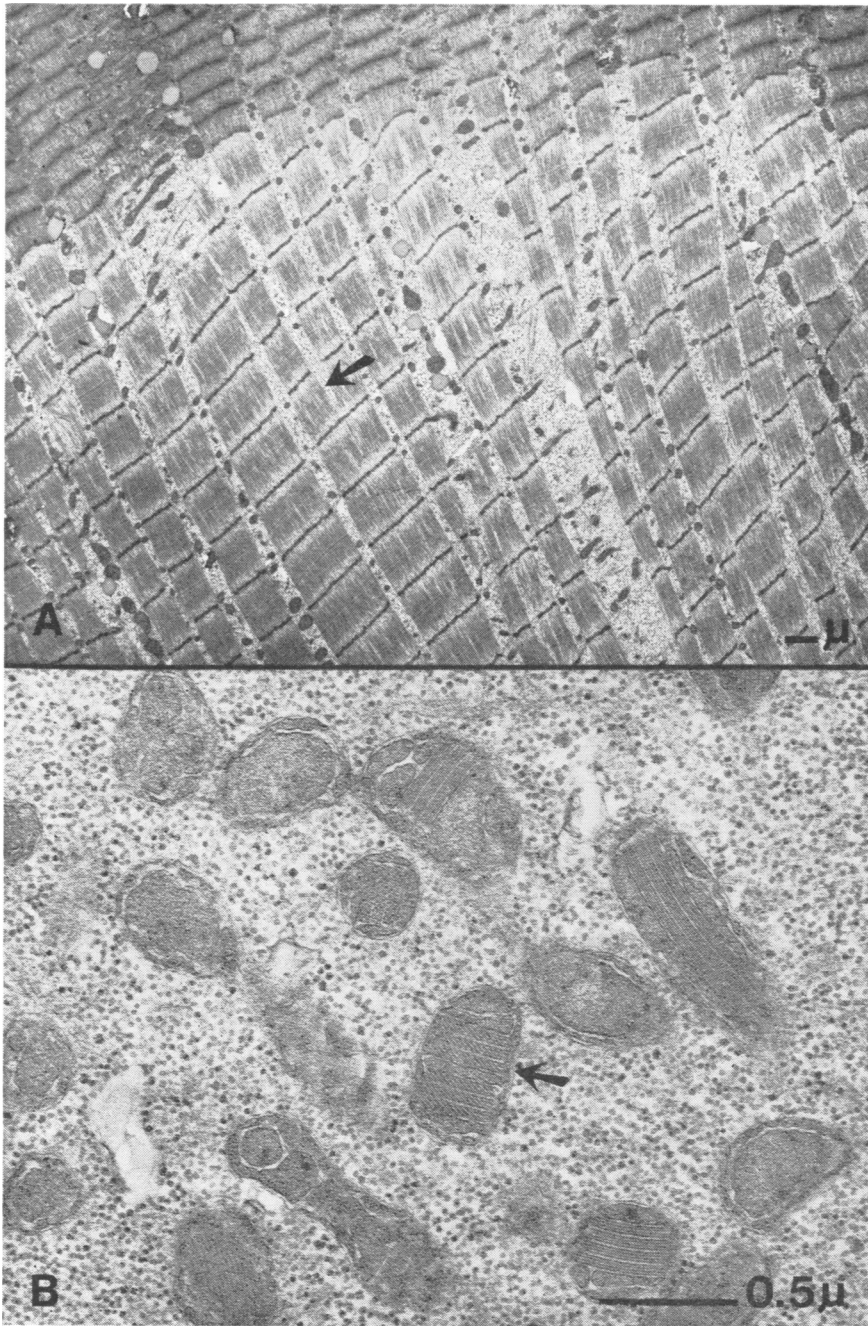


FIG. 5 *Limb-girdle muscular dystrophy*. A. From a 26 year old female. There is a wide separation of myofibrils and myofibrillar splitting (arrow) is evident. A focally contracted area is found in the upper part of the micrograph. B. From a 61 year old male. Intramitochondrial crystalline inclusions are present in many of these subsarcolemmal mitochondria. Some of the crystalline inclusions are located within the cristae (arrow).

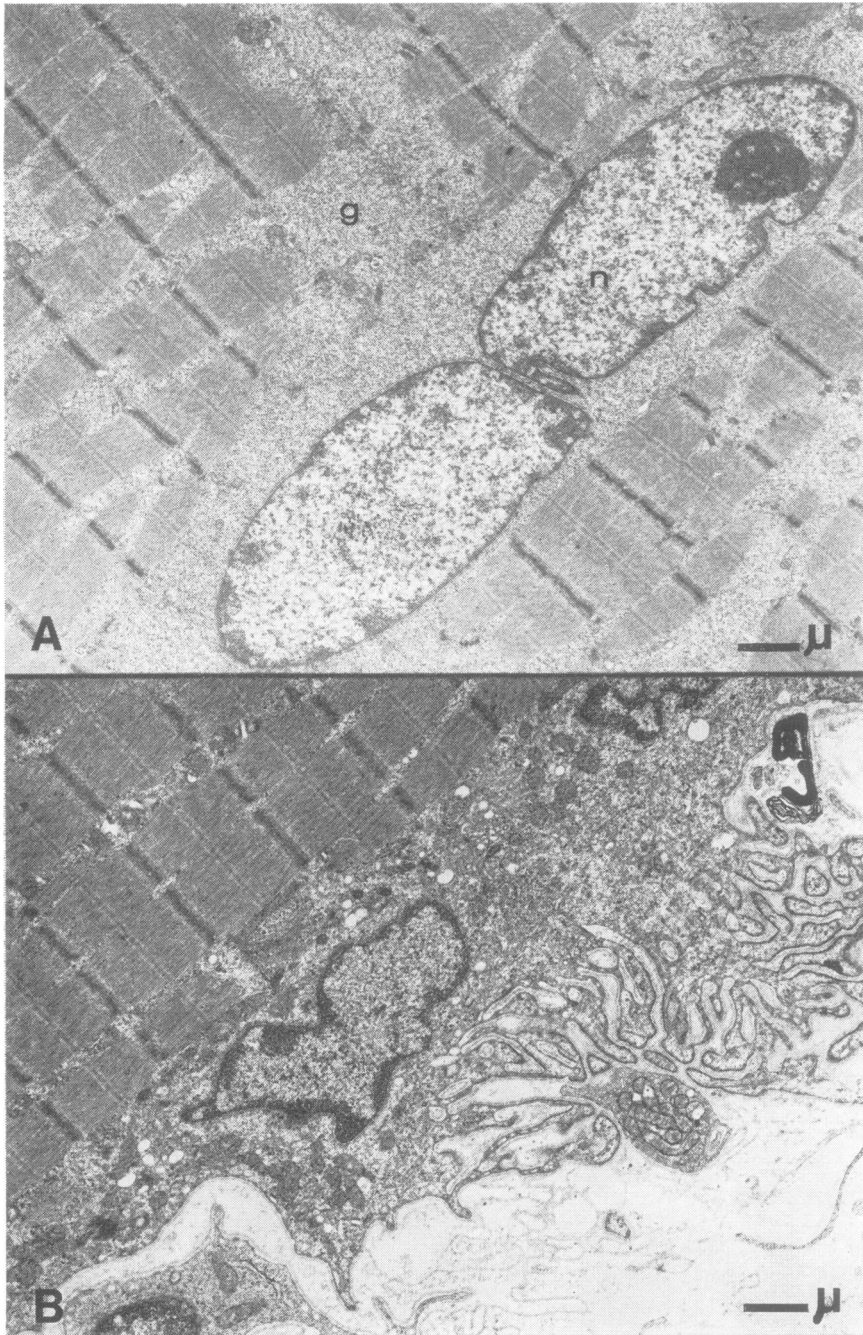


FIG. 6 Myotonic muscular dystrophy. **A.** From a 25 year old female. An internal nucleus (n) and an apparent increase in glycogen (g) are the only abnormalities present in this otherwise normal-looking muscle fibre. **B.** From a 46 year old female. Abnormal neuromuscular junction showing disorganization of the secondary synaptic clefts. Several myelin figures are present at the upper right of micrograph.

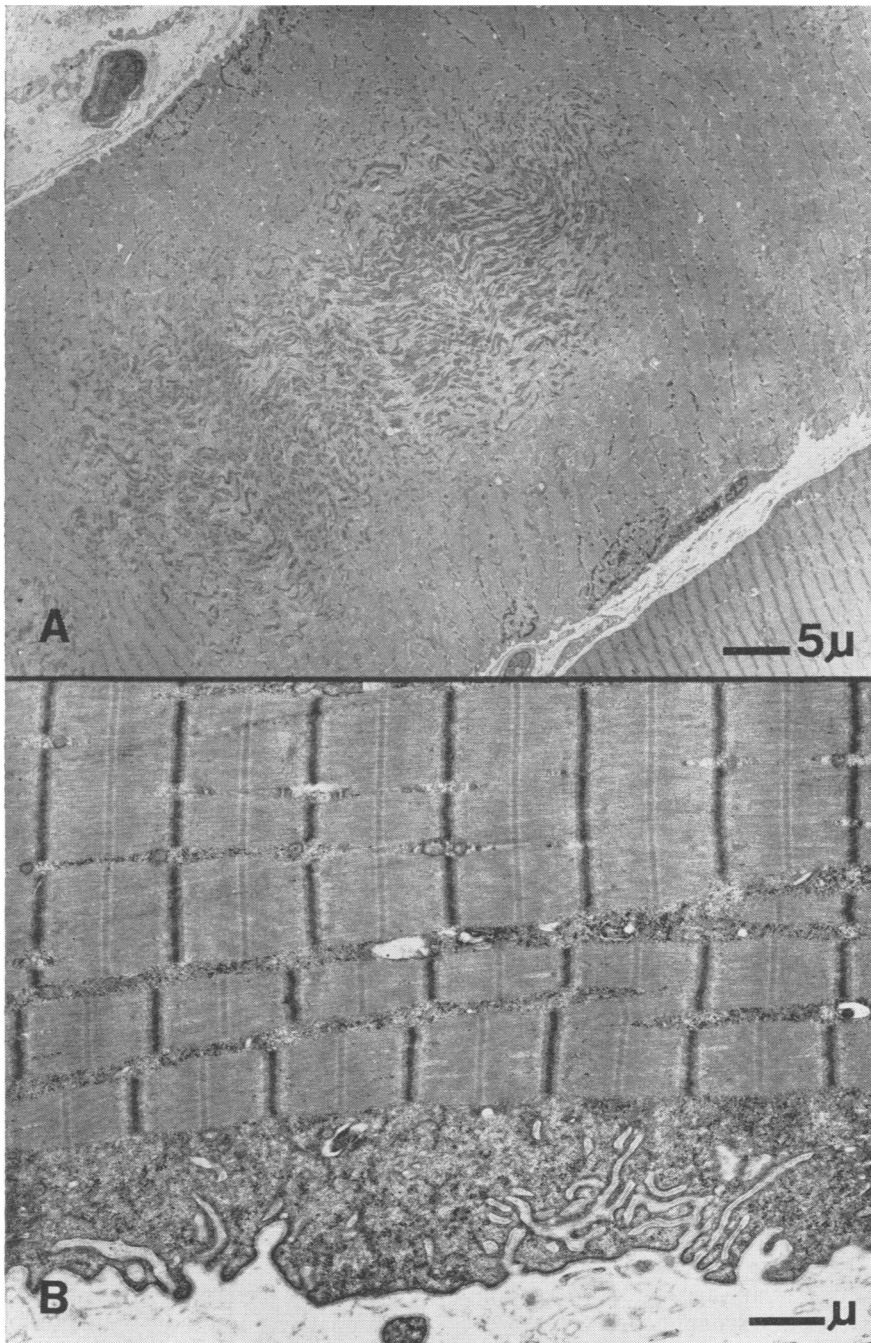


FIG. 7 *Motor neurone disease. A. From a 43 year old male showing a target-like fibre. B. From a 53 year old male showing invaginations of the sarcolemma which resemble in width the secondary synaptic clefts of a neuromuscular junction. No neural elements are seen in apposition to the synaptic clefts. The myofibrillar pattern appears relatively normal.*

finding in four of the five cases of marked type I fibre predominance (Fig. 2B). Moth-eaten fibres were more frequent in limb-girdle as compared with Duchenne type dystrophy. Biopsies from patients with myotonic dystrophy showed type I fibre atrophy (Fig. 2C) and, in some cases, type II hypertrophy as well. There was an increased number of internal nuclei but other architectural changes were usually mild.

Biopsies from patients with motor neurone disease showed small angular fibres, atrophy and hypertrophy of both fibre types, grouping of atrophic fibres, and some fibre type grouping (Fig. 2D). Occasional target fibres were also seen.

ELECTRON MICROSCOPY (Figs 3 to 7) The ultrastructural appearance of the biopsied normal intercostal muscles resembled that of normal adult human limb muscle (Fig. 3). Changes observed in the diseased muscles were similar to those previously described in biopsied limb muscle in each condition (Santa, 1969).

Biopsies from patients with Duchenne muscular dystrophy showed varying degrees of degeneration ranging from involvement of only a few sarcomeres to hyaline changes involving entire myofibrils (Fig. 4A). Degenerating muscle fibres often showed numerous vesicles, swollen mitochondria, and focal contractions. Satellite cells (Fig. 4B) were common and were seen even in minimally affected fibres. Biopsies from patients with limb-girdle dystrophy showed changes which were milder than those seen in the Duchenne form (Fig. 5A). Of note, however, was the finding of intramitochondrial inclusions in two of the five cases (Fig. 5B). These abnormal mitochondria were found in subsarcolemmal positions in otherwise normal appearing fibres as well as in atrophic ones. Biopsies from patients with myotonic dystrophy showed mild degenerative changes (such as Z-line smearing), internal nuclei, and increased glycogen (Fig. 6A). There were also abnormalities seen in all 14 of the neuromuscular junctions studied in this condition (Fig. 6B). The subneural apparatus in these cases showed varying degrees of randomness of the secondary synaptic clefts (compared with the normal parallel arrangement, *cf.* Fig. 3B), as well as abnormal length and width of these clefts. We did not see neuromuscular abnormalities in the nine junctions studied in

biopsies from patients with Duchenne dystrophy or in the 12 junctions studied in normal subjects.

Biopsy specimens from patients with motor neurone disease showed many atrophic and some necrotic fibres with areas of focal contraction, vesiculated mitochondria, and lipofuscin pigment accumulations. A target-like fibre with three characteristic zones is shown in Fig. 7A. Figure 7B, from another patient with motor neurone disease, shows a degenerated motor endplate. Degenerative changes were seen in each of the four neuromuscular junctions studied in this condition.

DISCUSSION

Despite its extensive use in investigations of normal and diseased human muscle, no detailed histochemical or electron microscopic study of biopsied external intercostal has been reported. We feel it is of interest, however, to compare our results with those obtained in previous studies of normal and abnormal human limb muscles.

We find that, in biopsied normal adult external intercostal muscle, histochemical type I and type II fibres are approximately of equal size, with both fibre types being larger in males than in females.

Brooke and Engel (1969) studied biopsied normal biceps brachii, vastus lateralis, deltoid, and gastrocnemius muscles. They found type II fibres in males to be generally larger than type I fibres, and the reverse to be true in females. Edström and Nyström (1969) evaluated biopsied normal biceps brachii and quadriceps muscles. They found, in both men and women, type II fibres to be relatively larger than type I fibres, but to a greater degree in the biceps.

Jennekens *et al.* (1971a) studied necropsy material and concluded that the differences in size of the fibre types were not the same when comparing normal deltoid, biceps brachii, rectus femoris, and gastrocnemius muscles. They found type I fibres to be relatively larger in the rectus femoris and gastrocnemius than in the deltoid and biceps. Conversely, type II fibres were found to be relatively larger in the deltoid and biceps brachii and in men compared with women.

With regard to fibre type percentages, we find equal proportions in normal male external intercostal muscle and a higher proportion of type I

fibres in the female. Brooke and Engel (1969) found a higher percentage of type II fibres in most of the normal limb muscle biopsies they studied, with the difference being greater in vastus lateralis and biceps brachii than in gastrocnemius and deltoid. They did, however, find that female biceps had a higher proportion of type I fibres than the male. Edström and Nyström (1969) also found a higher percentage of type I fibres in female than in male biceps brachii and, in the female, they counted more type I than type II fibres. Jennekens *et al.* (1971b) found a higher percentage of type I fibres in deltoid and gastrocnemius, more type II fibres in rectus femoris, and about 50% for each type in biceps brachii.

Our histochemical results in the diseased muscle group are consistent with those described in biopsied limb muscle (Dubowitz and Brooke, 1973). Each of the electron microscopic findings noted has also previously been reported in limb muscle biopsies from patients with the corresponding conditions. Thus, in Duchenne muscular dystrophy, numerous vesicles, swollen mitochondria, and focal contractions were described in peroneus brevis muscle by Santa (1969) while the occurrence of frequent satellite cells was reported in limb muscle by Shafiq *et al.* (1967). Subsarcolemmal intramitochondrial crystalline inclusions have previously been reported in a patient with limb-girdle dystrophy by Fardeau (1970). Abnormal neuromuscular junctions were described in limb muscle from patients with myotonic dystrophy by Allen *et al.* (1969). Target fibres have been described ultrastructurally in limb muscle in motor neurone disease by Schotland (1969). Lipofuscin accumulations have been reported in limb muscle of patients with this disorder by Shafiq *et al.* (1967) and Mumenthaler (1970) described grossly abnormal changes in limb muscle, similar to those observed here.

In summary, we found that normal adult human external intercostal muscle showed a mosaic pattern of histochemical type I and type II fibres, with equal percentages in males and more type I fibres in females. The two fibre types were of about equal diameters but both were larger in men than in women. In patients with several forms of muscular dystrophy and motor neurone disease, the morphological

changes observed resembled those previously described in biopsied limb muscle and underline the particular usefulness of the intercostal preparation.

REFERENCES

- Allen, D. E., Johnson, A. G., and Woolf, A. L. (1969). The intramuscular nerve endings in dystrophia myotonica—a biopsy study by vital staining and electron microscopy. *Journal of Anatomy*, **105**, 1–26.
- Brooke, M. H., and Engel, W. K. (1969). The histographic analysis of human muscle biopsies with regard to fiber types. 1. Adult male and female. *Neurology (Minneapolis)*, **19**, 221–233.
- Brooke, M. H., and Kaiser, K. K. (1970). Muscle fiber types: how many and what kind? *Archives of Neurology*, **23**, 369–379.
- Creese, R., Dillon, J. B., Marshall, J., Sabawala, P. B., Schneider, D. J., Taylor, D. B., and Zinn, D. E. (1957). The effect of neuromuscular blocking agents on isolated human intercostal muscle. *Journal of Pharmacology and Experimental Therapeutics*, **119**, 485–494.
- Dubowitz, V., and Brooke, M. H. (1973). *Muscle Biopsy: A Modern Approach*. Saunders: London.
- Edström, L., and Nyström, B. (1969). Histochemical types and sizes of fibres in normal human muscles. *Acta Neurologica Scandinavica*, **45**, 257–269.
- Elmqvist, D., Hofmann, W. W., Kugelberg, J., and Quastel, D. M. J. (1964). An electrophysiological investigation of neuromuscular transmission in myasthenia gravis. *Journal of Physiology*, **174**, 417–434.
- Elmqvist, D., Johns, T. R., and Thesleff, S. (1960). The study of some electrophysiological properties of human intercostal muscle. *Journal of Physiology*, **154**, 602–607.
- Elmqvist, D., and Quastel, D. M. J. (1965). A quantitative study of end-plate potentials in isolated human muscle. *Journal of Physiology*, **178**, 505–529.
- Engel, W. K., and Cunningham, G. G. (1963). Rapid examination of muscle tissue. An improved trichrome method for fresh-frozen biopsy sections. *Neurology (Minneapolis)*, **13**, 919–923.
- Farber, E., Sternberg, W. H., and Dunlap, C. D. (1956). Histochemical localization of specific oxidative enzymes. *Journal of Histochemistry and Cytochemistry*, **4**, 254–263.
- Fardeau, M. (1970). Ultrastructural lesions in progressive muscular dystrophies. A critical review of their specificity. In *Muscle Diseases*, pp. 98–108. Edited by J. N. Walton, N. Canal, and G. Scarlato. Excerpta Medica: Amsterdam.
- Gruener, R., Stern, L. Z., Payne, C. M., and Hannapel, L. K. (1975). Hyperthyroid myopathy: Intracellular electrophysiological measurements in biopsied human intercostal muscle. *Journal of the Neurological Sciences*, **24**, 339–349.
- Hofmann, W. W., Birnberger, K. L., Harlacher, A., and Schreiber, M. (1973). Normal and dystrophic human muscle *in vitro*: an argument against denervation. *Experimental Neurology*, **39**, 249–260.
- Jennekens, F. G. I., Tomlinson, B. E., and Walton, J. N. (1971a). The sizes of the two main histochemical fibre types in five limb muscles in man. *Journal of the Neurological Sciences*, **13**, 281–292.
- Jennekens, F. G. I., Tomlinson, B. E., and Walton, J. N. (1971b). Data on the distribution of fibre types in five human limb muscles. *Journal of the Neurological Sciences*, **14**, 245–257.
- Ludin, H. P. (1969). Microelectrode study of normal human skeletal muscle. *European Neurology*, **2**, 340–347.

- Ludin, H. P. (1970). Microelectrode study of dystrophic human skeletal muscle. *European Neurology*, **3**, 116-121.
- Mumenthaler, M. (1970). Myopathy in neuropathy. In *Muscle Diseases*, pp. 585-598. Edited by J. N. Walton, N. Canal, and G. Scarlato. Excerpta Medica: Amsterdam.
- Padykula, H. A., and Herman, E. (1955). The specificity of the histochemical method of adenosine triphosphatase. *Journal of Histochemistry and Cytochemistry*, **3**, 170-195.
- Santa, T. (1969). Fine structure of the human skeletal muscle in myopathy. *Archives of Neurology*, **20**, 479-489.
- Santa, T., Engel, A. G., and Lambert, E. H. (1972a). Histo-metric study of neuromuscular junction ultrastructure. 1. Myasthenia gravis. *Neurology (Minneapolis)*, **22**, 71-82.
- Santa, T., Engel, A. G., and Lambert, E. H. (1972b). Histo-metric study of neuromuscular junction ultrastructure. 2. Myasthenic syndrome. *Neurology (Minneapolis)*, **22**, 370-376.
- Schotland, D. L. (1969). An electron microscopic study of target fibers, target-like fibers and related abnormalities in human muscle. *Journal of Neuropathology and Experimental Neurology*, **28**, 214-228.
- Shafiq, S. A., Gorycki, M. A., and Milhorat, A. T. (1967). An electron microscopic study of regeneration and satellite cells in human muscle. *Neurology (Minneapolis)*, **17**, 567-574.
- Spurr, A. R. (1969). A low-viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructure Research*, **26**, 31-43.
- Stern, L. Z., Payne, C. M., and Hannapel, L. K. (1974). Acromegaly: Histochemical and electron microscopic changes in deltoid and intercostal muscle. *Neurology (Minneapolis)*, **24**, 589-593.