The syndrome of 'continuous muscle-fibre activity' cured: further studies

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SYNOPSIS Two cases suffering from the syndrome of 'continuous muscle-fibre activity' have been followed-up for 14 years. These patients have gradually gone into remission and no longer require therapy. The results of recent histology, histochemistry, and electronmicroscopy, as well as sural nerve biopsy studies, are presented. The sarcoplasmic reticulum calcium binding activity and ATPase activity are normal.

In 1961 two cases of 'continuous muscle-fibre activity' were described (Isaacs, 1961). The typical features of this syndrome served to separate it from other known forms of neuromuscular disease. Since this time, a number of other typical cases have been described by Levy *et al.* (1965), Mertens and Zschocke (1965), Isaacs (1967), Buscaino *et al.* (1970), Desai *et al.* (1970), Wallis *et al.* (1970), and Welch *et al.* (1972). In 1960 after prolonged investigation diphenylhydantoinate was found to offer dramatic symptomatic relief from the continuous muscle-fibre activity. It was furthermore sug-

gested that the muscle-fibre activity occurred in response to an abnormal release of acetylcholine by the motor nerve terminals.

The original two cases have now been followed-up for 14 years and over the past few years it was noticed that the manifestations of the disease were becoming less troublesome. Smaller doses of diphenylhydantoinate were needed until the present time when treatment is no longer necessary. The clinical improvement relates well with the muscle histology and histochemistry and these features are presented. The sural nerves have been biopsied, as it was sug-

FIG. 1 (left). Showing increased variability of the size of muscle fibres. There is an increase in the number of central nuclei and several of the nuclei are larger than normal. $\times 35$. FIG. 2 (right). Myofibrillar ATPase stain showing an area of excessive grouping of the type I fibres. $\times 35$.

gested by Welch *et al.* (1972) that sensory nerves may also be involved. In addition, the relaxing capacity of the isolated sarcoplasmic reticulum has been studied as well as actomyosin adenosine-triphosphate.

METHODS

Muscle tissue was removed at various times from three patients suffering from this disease. The muscles sampled included the pectoralis, vastus lateralis, peroneus longus, anterior tibial, and the deltoids. The histochemical and histological profiles included NAD diaphorase, myofibrillar ATPase, mitochondrial ATPase, phosphorylase, PAS stain for glycogen, modified trichrome stains, and routine haematoxylin and eosin (H and E) stains.

The sural nerve segments were removed under local anaesthesia, maintained at resting length, and fixed in both formalin and osmic acid. Single fibre preparations were obtained with the use of the dissecting microscope after preparation in glycerine. Transverse and cross sections of nerve were cut after wax embedding and stained with H and E. Muscle was also biopsied at the same time for electron-microscopic study. This muscle was held in clamps to maintain the resting length and fixed in glutaraldehyde, processed, and then embedded in Epon. Thin sections were cut and examined with a Siemens Einskop electronmicroscope.

Sarcoplasmic reticulum was isolated from biopsy specimens of the m. vastus lateralis of one patient and muscle from three normal individuals was used as controls. The isolation procedure was essentially

according to the method of Duggan (1971). Muscle was homogenized in 9 volumes of 0.3 M sucrose 20 mM imidazole-HCl, pH 6.85, with an all-glass hand homogenizer at 2°C. The homogenate was centrifuged at 2,000 *g* for 10 minutes to sediment the myofibrillar fraction, which was then kept for extraction of actomyosin. The supernatant fluid was centrifuged twice at 15,000 *g* to remove the mitochondria. Sarcoplasmic reticulum was sedimented by centrifuging the supernatant at 200,000 *g* for 60 minutes, and resuspended in 1.0 ml homogenizing medium per gram of original tissue. The above procedures were carried out at 2°C. ATPase and Ca²⁺ uptake studies were made immediately after preparation of the sarcoplasmic reticulum using the millipore filtration procedure of Martonosi and Feretos (1964), and with the same substrate and protein concentrations described by Samaha and Gergely (1965). The pH of the assay medium was 6.85 and activities were measured at 37°C. The assay medium contained 0.1 mM ⁴⁵CaCl₂ (0.5 µCi/ml). (The rate of Ca²⁺ binding was measured after one minute's reaction, and calcium uptake (total) after 10 minutes' reaction in the assay medium.) Calcium uptake was computed by the method of Sylvester and Baskin (1971). ATPase activity was measured as inorganic phosphate liberated by the method of Taussky and Shorr (1953).

Actomyosin ATPase was extracted from the myofibrillar fraction, mentioned above, in 5 volumes of 0.6 M KCl-25 mM tris-HCl, pH 7.3 for 24 hours at 2°C, after which it was purified by two cycles of centrifugation at 20,000 *g* for 30 minutes and precipitation by dilution. Actomyosin ATPase activity was measured at 30°C essentially according

FIG. 3 (left). Myofibrillar ATPase stain showing a reasonably normal distribution of muscle fibre types. $\times 35$.
FIG. 4 (right). There is less variability in fibre size and central nuclei are less evident. Haematoxylin and eosin preparation. $\times 35$.

FIG. 5. Cross-section of the sural nerve showing a normal distribution of axons. Osmium tetroxide and haematoxylin and eosin preparation. $\times 160$.

FIG. 6. Electron-micrograph showing normal myofibrils with a centrally situated nucleus. $\times 4,000$.

to Heffron and Duggan (1971) in the following assay media: abpH 7.3: Mg^{2+} -ATPase: 5 mM ATP-tris, 5 mM $MgCl_2$, $u=0.05$; Ca^{2+} ATPase: 5 mM ATP-tris, 5 mM $CaCl_2$, $u=0.5$; EDTA-ATPase: 5 mM ATP-tris, $u=0.5$. Protein was estimated by the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

RESULTS

The muscle histology and histochemistry over the past few years has shown that the abnormal variation in fibre size (Fig. 1) as well as the increased grouping of individual fibre types (Fig. 2) has become less obvious (Fig. 3). The numerous

TABLE 1
CALCIUM UPTAKE AND ATPase ACTIVITY IN THREE NORMAL SUBJECTS AND PATIENT

	Calcium uptake				ATPase activity	
	No oxalate	5 mM oxalate	No oxalate	5 mM oxalate	No oxalate	Oxalate
	Initial rate		(μmol/mg protein in 10 min)		(μmol Pi/mg protein/min)	
	(μmol/mg protein/min)					
Normal (3)	0.233 ± 0.011	0.790 ± 0.049	0.205 ± 0.018	1.958 ± 0.103	0.200 ± 0.012	0.676 ± 0.021
Patient	0.218	0.696	0.138	1.510	0.118	0.411

Data are expressed as mean ± SEM. All activities measured at 37°C.

nuclei of degenerating and regenerating fibres are now few (Fig. 4). The sural nerves were found on both single fibre and cross-sectional studies to be normal (Fig. 5). The recent electron-microscopic study of muscle removed from the vastus lateralis was within normal limits (Fig. 6).

The initial rate, total uptake of calcium, and ATPase activities of sarcoplasmic reticulum from

oxalate, was reduced by 37 and 34%, respectively, compared with the lower limit of the normal range. There was no real difference in the yield of sarcoplasmic reticulum from the patient and the controls, the values being 1.90 and 2.05 ± 0.06 mg of sarcoplasmic protein per gram of fresh tissue, respectively.

The Mg²⁺- and EDTA-activated ATPase activities of actomyosin were the same in the patient and the control, while a small decrease was observed in the case of the Ca²⁺-activated enzyme (Table 2).

TABLE 2
ACTOMYOSIN ATPase ACTIVITIES OF NORMAL AND PREVIOUSLY DISEASED MUSCLE

	ATPase activity Mg ²⁺ -ATPase	Ca ²⁺ -ATPase	EDTA-ATPase
		(μmol Pi/mg protein/min)	
Normal (3)	0.239 ± 0.017	0.208 ± 0.021	0.647 ± 0.045
Patient	0.241	0.149	0.715

Data are expressed as mean ± SEM. Enzyme activities measured at 30°C.

the patient and three normal individuals are shown in Table 1. The initial rate of calcium binding by the sarcoplasmic reticulum in the presence and absence of 5 mM oxalate, measured after one minute's reaction, is essentially the same in the controls and the patient, the normal ranges being 0.711–0.882 and 0.245 μmol Ca/mg protein/min, respectively. However, the total uptake of calcium in the presence and absence of oxalate was decreased by 17 and 25%, respectively, when referred to the lower limit of the normal ranges. The Mg²⁺-activated ATPase activity of the sarcoplasmic reticulum of the patient, measured in the presence and absence of

DISCUSSION

The site of the abnormal discharge was localized to the motor nerve terminals (Isaacs, 1961, 1964, 1967). It is, nevertheless, possible that some of the abnormal activity originates in more proximal sites of the motor nerves (Welch *et al.*, 1972). The reduced sensory axonal population in the sural nerve in the patient of Welch *et al.* (1972) led them to believe that sensory involvement was also present in this syndrome. This finding, however, was contrary to the clinical and electrophysiological findings and as Arnold and Harriman (1970) have pointed out the sural nerve findings are often misleading. Further to investigate this finding, sural nerve biopsies were taken from both the original patients and found to be entirely normal (Fig. 5).

Over the passage of 14 years spontaneous gradual cure has occurred and this has been accompanied by improvement in the muscle histology and histochemistry. Fibre type grouping, in particular, is now far less evident. We realize that it is impossible to have accurate controls with muscle biopsy tissue, as the same

area cannot be re-examined, but the changes over the past 14 years suggest that the earlier peripheral involvement of the motor nerves having subsided has allowed the nerves to resume their normal stimulatory and trophic activity and reclaim their motor unit territory. The possibility of reclaiming motor unit territory has no experimental backing but it has been obvious from earlier motor end-plate and nerve terminal studies that double innervation of a single muscle fibre was common in this condition and so perhaps it is a question of re-establishing a physiological preponderance.

As far as the present biochemical studies are concerned, it is clear from the data presented that the initial rate of calcium binding is normal, indicating that the rate of relaxation is also normal. The small reduction in total calcium uptake observed may not be significant in view of the small number of controls tested. Although the Mg^{2+} -activated ATPase activity of the sarcoplasmic reticulum of the patient was reduced, the ratio of initial rate of calcium binding to ATPase activity is slightly higher (Table 1). Thus the efficiency of calcium binding is not altered in the patient, suggesting that the relaxing mechanism is intact. Similarly, the content of sarcoplasmic reticulum in the muscle of the patient, measured as total protein in the isolated reticulum is normal. The contractile apparatus is intact as shown by the normal actomyosin ATPase activity of the patient. Thus the present clinical, histological, and histochemical findings are in general accord with the biochemical data, indicating that the patients' muscles have recovered almost completely. The eventual recovery of these patients gives no clue to the initial causative mechanism but tends to rule out an inherited abnormality.

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