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Relationship of spontaneous fibrillation potentials to muscle fibre segmentation in human muscular dystrophy

DENERVATED skeletal muscle fibres have long been known to show spontaneous fibrillation potentials, this being one of the features resulting from the removal of the trophic influence of the motor nerve[1]. Electromyography (EMG) has shown spontaneous fibrillations in human myopathies such as myositis or muscular dystrophy[2–6], but their true incidence and mechanism is still obscure. We propose that myopathic fibrillations result from segmental necrosis of muscle fibres so that a distal fibre segment is separated from the part carrying the motor endplate. We show here, first, that after experimental myotomy in the baboon biceps muscle, the nerve-free segments develop fibrillation after a consistent delay and second, that by comparing different clinical types of human myopathies a correlation can be found between the incidence of focal necrosis and spontaneous fibrillation potentials. Our findings are consistent with our previous report of collateral innervation of newly formed nuscle fibres in Duchenne muscular dystrophy[7].

Experimental myotomies were performed in sterile conditions in seven healthy adult baboons or macaques, under light pentothal anaesthesia. EMG tests and biopsies were then carried out and the animals were not killed. The biceps brachii muscle was used because its motor endplates are concentrated in a narrow innervation zone[8] which can be identified on the exposed muscle by focal stimulation with a sterile steel electrode placed on the superficial muscle fibres. Threshold current pulses of 0.1 ms duration (about 1 mA) elicited brisk twitches at the motor point but the same stimuli were ineffective when sited more distally on the same bundles (Fig. 1*a*). A number of small cuts were made in several muscle bundles with iridectomy scissors, at least 10–15 mm distal to the motor point thus defined. We were careful not to cause any lesion to the blood supply. The aponeurosis and skin were sutured. One to two months after the physiological observations a muscle biopsy was removed and longitudinal sections 5–10 μm were made. Staining for cholinesterase by the acetylthiocholine method disclosed motor endplates at the level of the previously identified motor point (Fig. 1*b*) whereas no focal cholinesterase accumulation was found throughout the distal part of the biopsy (Fig. 1*c*). Obviously the myotomies failed to involve any motor nerve fibre because they were quite distal to the innervation zone and intramuscular nerves. Some biopsy specimens were also stained with Masson trichrome, showing fairly normal isolated muscle fibre segments at the level of the myotomy (Fig. 1*d*). The segments had restored their membrane at the level of the lesion where myoblast proliferation and myotube formation were seen, with typical vesicular nuclei containing prominent nucleoli (Fig. 1*e*).

After the operation the animals were tested repeatedly after receiving a small intramuscular dose of tranquiliser (Sernylan or Thalamonal). The biceps muscles were explored with a sterile concentric needle electrode inserted through the skin, as for a human EMG. No fibrillation potentials were observed in the first 5 d. After 5 to 6 d, abundant fibrillations of typical triphasic waveform were recorded in many sites of the operated biceps, but only distally with respect to the myotomies (Fig. 2). No fibrillation was detected above that level where typical motor unit potentials could be elicited by stretching the muscle slightly. The presence of the needle electrode in the muscle did not trigger the fibrillation potentials because the latter could readily be demonstrated with subcutaneous steel needles which did not enter the muscle itself (Fig. 2*a*). A few of the muscles were not tested before the tenth day to ensure that the fibrillations then recorded could not be ascribed to an inadvertent lesion possibly related to electrode insertions. The distal muscle fibre segments which fibrillated had obviously been deprived of neural influence since the time of the mytomy. On the other hand, when muscle fibres are denervated by neurotomy, the distal nerve stump takes about 2–3 d to degenerate in the baboon[10] and it would be expected to maintain some trophic action on the muscle during this time[11]. Two animals were prepared with biceps myotomy on one side and with a partial section of the biceps nerve on the other side. Spontaneous fibrillations started in the distal biceps with myotomy about 2 d earlier than in the partially denervated biceps. About 100 different fibrillation potentials were recorded after 10–20 d on either side in these animals. Most potentials were triphasic and their mean total duration was 3.1 ± 1.0 ms. The mean voltages were 136 ± 76 μV on the myotomy side and $112 + 53$ μV on the partially denervated side ($P < 0.01$). Whereas in devervated muscle fibres, fibrillations generally arise at the site of the old endplate[12] this is not an absolute rule[1] and our results indicate that fibrillations are generated in nerve-free segments of primate muscle (Fig. 2). Other changes characteristic of removal of neural trophic factors had been found in frog muscle segments—the appearance of acetylcholine receptors[13] and the acceptance of motor innervation[14].

The hypothesis that myopathic fibrillations result from muscle fibre segmentation is further supported by the finding that spontaneous fibrillations are much more abundant than hitherto suspected in Duchenne muscular dystrophy[4,15], whereas they were very scarce, if present at all, in the muscles of five patients with typical facio-scapulo-humeral (Landouzy–Dejérine) muscular dystrophy. The most important pathological change in Duchenne muscular dystrophy is focal necrosis which isolates long fibre segments from the endplate region[16,17] whereas this lesion is rare in facio-scapulo-humeral muscular dystrophy[17]. The correlation also holds for the muscles of patients with acute polymyositis which have marked lesions of focal necrosis[18] as well as abundant fibrillations in EMG tests. Because they generally looked at transverse muscle sections most pathologists failed to emphasise that myopathic necrotic lesions, far from destroying the muscle fibres, result in serially arranged fibre segments. That nerve-free segments thus formed are collaterally innervated and can then be activated by the motor nerve in Duchenne muscular dystrophy[7,15], and also in polymyositis[19], sheds new light on the pathogenesis and emphasises regenerative capabilities which must retard the clinical progression and/or promote the recovery of human myopathies with muscle fibre segmentation.

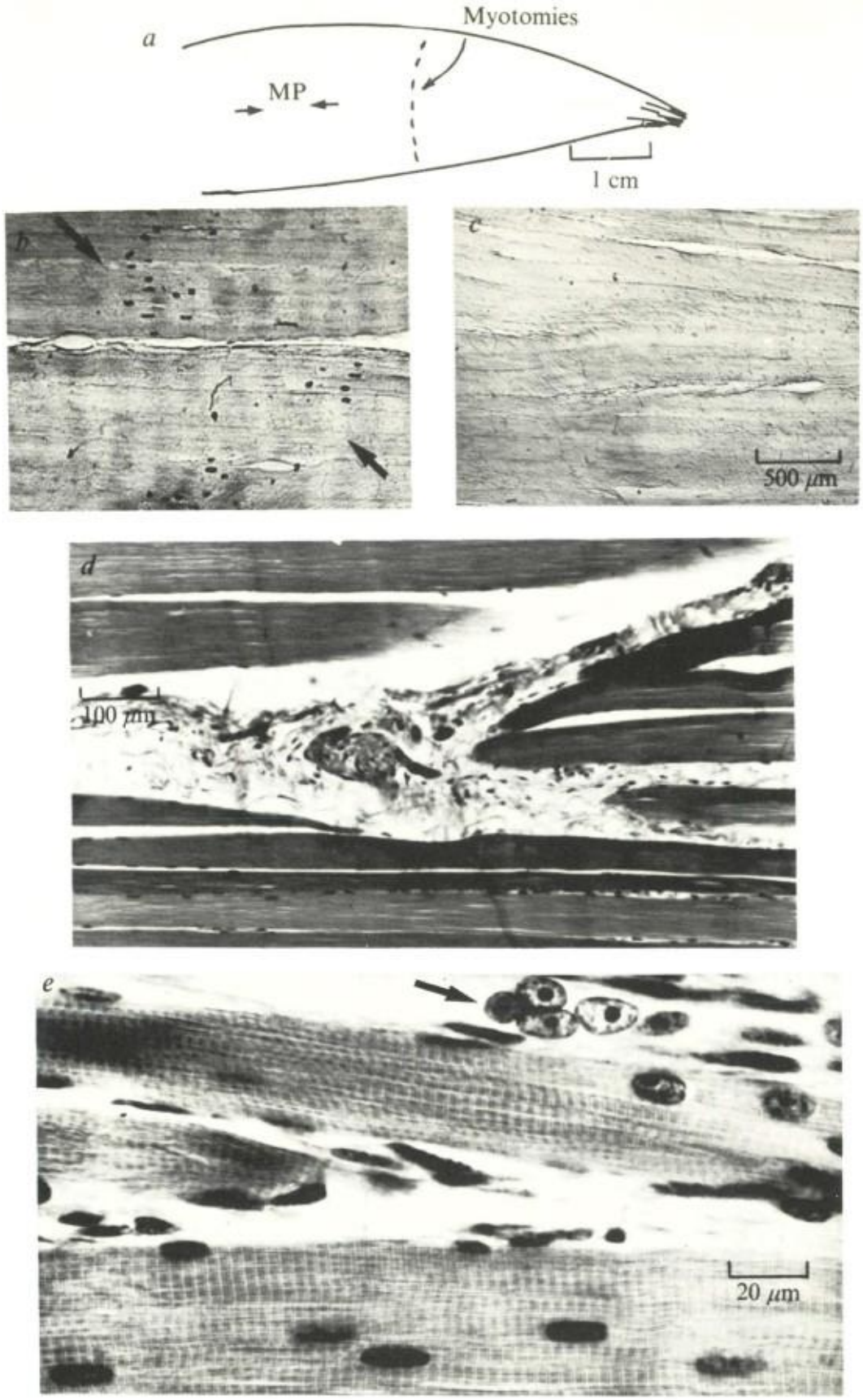

Fig. 1 Longitudinal histological sections in baboon biceps biopsied 2 months after extrajunctional myotomies. *a*, Sketch of the muscle with the motor point (MP) located by electrical stimulation and the distal myotomies. *b* and *c*, Acetylthiocholine method for staining motor endplate cholinesterase. *b*, Motor point region with many endplates (between arrows). *c*, Distal biceps near myotomies with no cholinesterase accumulations. *d* and *e*, Sections through myotomy area stained with Masson trichrome. *d*, Segmented muscle fibres (middle) next to intact muscle fibres (below). *e*, Myotube with four myoblasts (arrow).

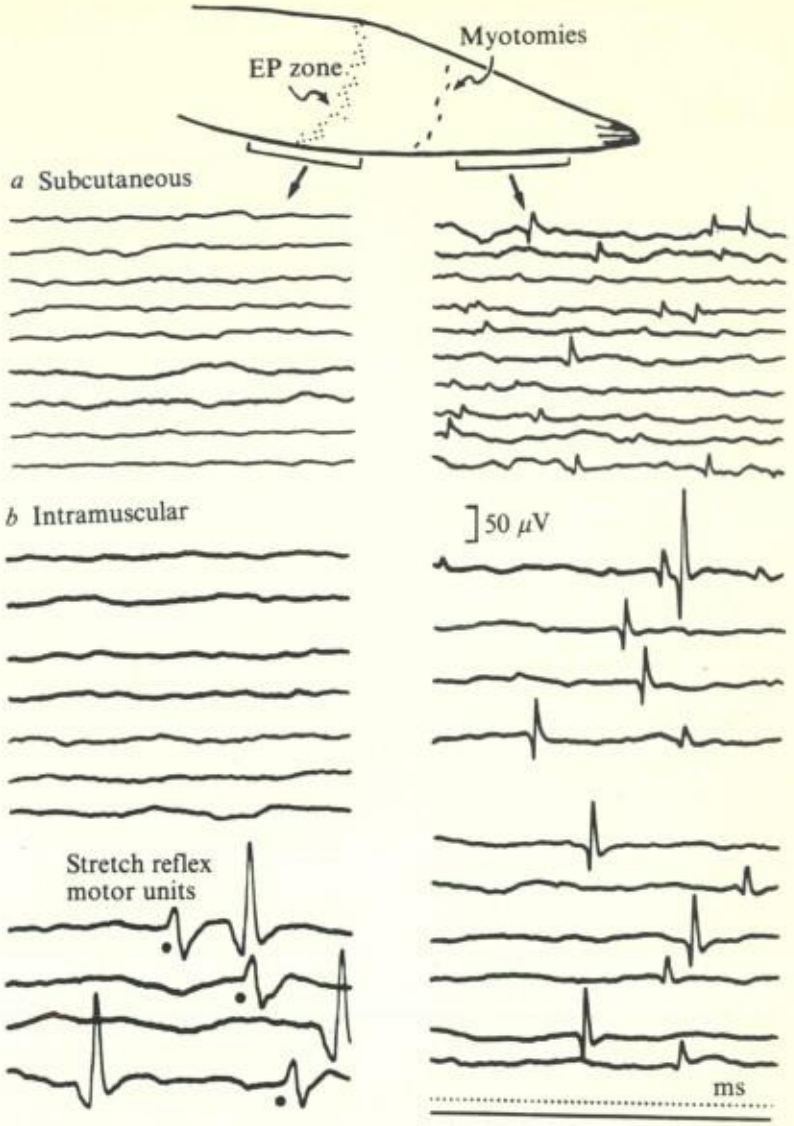

Fig. 2 Cathode ray oscillograms from baboon biceps muscle, 10 d after extrajunctional myotomy. Recording from the motor endplate zone (left column) and from the region distal to myotomy (right column). *a*, Subcutaneous bipolar needles not penetrating into the muscle. *b*, Concentric needle electrode in the muscle. Same calibration for all records. Fibrillation potentials are only recorded in distal region. At the bottom of the left column, a slight stretch elicited motor unit potentials and the one marked by dots starts with a negative (upwards) component which indicates focal recording at the endplate (see refs 8 and 9).

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