Fiber Lengths and End-plate Locations in Fruit Bat Web Muscles* (32801)

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Web muscles of the East African fruit bat have been used to demonstrate skeletal muscle regeneration (1,2). Interpretation of such results would be facilitated by measurement of web muscle fiber lengths. Consequently, in this study lengths of single muscle fibers, as well as the location of end-plates on fibers, have been determined both electrically and histologically.

Materials and Methods. Eleven fruit bats (*Eidolon helvum* Kerr) were kept in a single cage and fed ripe bananas. Bats were handled with thick leather gloves to protect the experimenter from bites. They were relaxed with an intramuscular injection of chlorpromazine hydrochloride (Largactil, 2.5 mg/100 gm of body wt.) 30 min before being mounted in a supine position on a Lucite board with the wings extended and held firmly (1). Bats were lightly anesthetized with ether dropped on a face mask.

Electrical recording. One of the 13–15 web muscles in the ulnar group and its branch of the web nerve were exposed by removing the ventral skin. Exposed tissues were kept wet with Locke's solution at room temperature (about 24°C), and the nerve bundle was dissected down to 1–5 nerve fibers immediately medial to the muscle being studied. Insulated paired steel electrodes with tips 285 μ apart were used to stimulate the cut-down motor nerve. Stimuli of 0.2-msec duration and just supramaximal amplitude (3–10 V) were applied at a usual rate of 1/sec. Extracellular recording electrodes with tips 250 μ apart were connected through a high-gain differential amplifier to an oscilloscope (Solartron CD 1400). Recording from only one muscle fiber was facilitated by the wide distribution through the muscle of the estimated 40–50 muscle fibers per motor unit. The three criteria used for responses of a single muscle fiber were as follows: (i) an abrupt threshold with no increase in amplitude of the muscle action potential with increased stimulating voltage, (ii) all-or-none failure after a brief period of tetanic stimulation (100/sec) without any irregularities in the action potential, and (iii) a constant refractory period when tested with paired stimuli. Diphasic action potentials were recorded along the length of a muscle fiber, while monophasic action potentials appeared and were photographed when recording at the ends of a fiber. There were no electrical responses beyond the two ends of a fiber. End-plates were located by the electrode position yielding action potentials of minimum latency.

Isolated fiber measurement. Twenty muscles including the tendons at both ends were cut from three webs of two bats and were immersed in 20% nitric acid to dissolve away fibrous connective tissue (3). Solutions of trypsin or papain were ineffective. After 6 hours both ventral and dorsal layers of skin were peeled off, and bare muscles were transferred into Locke's solution. Single muscle fibers were teased free with a pair of fine pins and removed to a microscope slide. After the fluid had dried the fiber was observed under oil immersion. Muscle fibers were judged intact if they had gradual tapers at both ends. Muscle fiber length was measured by moving the microscope stage along one coordinate axis and reading its vernier scale to the nearest 0.1 mm.

End-plate staining. Seven muscles were removed individually from one web, and the subneural apparatus was stained for "lead-reactive substance" (4). Whole muscles were immersed in 5% lead nitrate solution for 20 min, followed by immersion in 2% sodium sulfide solution for 15 min. After staining

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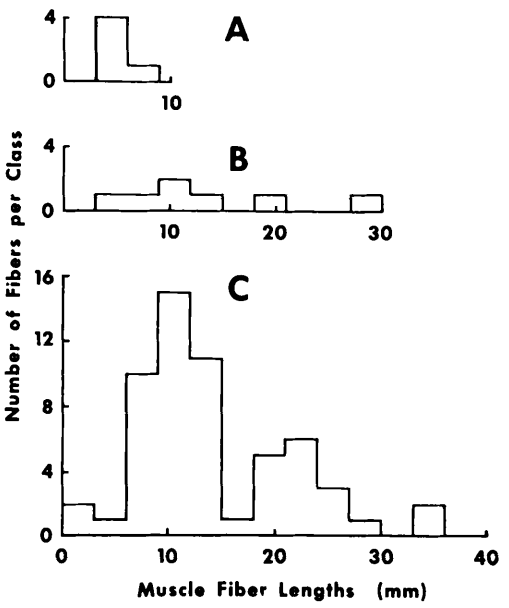

FIG. 1. Distributions of muscle fiber lengths. Lengths in classes of 3 mm (abscissa) plotted against the number of muscle fibers observed in each class (ordinates). A: electrical measurements of non-terminal fibers; B: electrical measurements of terminal fibers; C: morphological measurements of predominantly terminal fibers.

muscles were treated with nitric acid to dissolve connective tissue, and single muscle fibers were isolated, although only 1 of 15 fibers remained unbroken. Fiber lengths were measured, and the location of the end-plate in relation to both ends of the muscle fiber was recorded.

Results. The mean length of 12 muscle fibers determined by electrical recording was 10.1 mm. Frequency distributions of muscle fiber lengths are shown in Fig. 1. Seven fibers with one end attached to a muscle tendon had a mean length of 13.5 mm (Fig. 1B), while 5 fibers without such attachment had a mean length of 5.2 mm (Fig. 1A). This difference suggests that fibers may be longer at the ends of muscles.

The end-plate position was a mean of 39% of total fiber length from one end in those 6 of the 12 fibers with complete records (range of 27–48%). This demonstrates that end-plates are near the middle of muscle fibers although not precisely medial.

The mean length of 57 intact isolated muscle fibers was 14.1 mm with a range of 0.5–35.5 mm. The frequency distribution of lengths (Fig. 1C) is bimodal with peaks at 10 and 21 mm, although it may be a skewed normal distribution with a chance lack of fiber lengths near 16 mm.

Staining also demonstrated that end-plates were near the middle of muscle fibers (left side of Table I). In partly damaged fibers all end-plates were closer to the broken tip than to the intact tip, which supports the medial location of end-plates. End-plate locations are representative even in fibers with both tips broken, since their lengths were only slightly reduced from the length of one intact fiber (right side of Table I) or in comparison with other isolated fibers (Fig 1C).

Discussion. The lengths of intact isolated muscle fibers span the values for fibers determined by electrical recording (Fig. 1). Fibers measured by electrical responses were on the average shorter, but the larger series of isolated fiber measurements is probably more representative. Tenuissimus muscles in the rat are similar in dimensions and fiber length to bat web muscles. They measure 9–15 cm long and 2–4 mm wide with about 1000 fibers in cross-section (3). In comparison, bat wing web muscles are 1–10 cm long and 1–3 mm wide with 600–800 fibers in cross-section (J. C. T. Church, personal communi-

TABLE I. Location of Stained Endplates on Single Muscle Fibers.

Condition of fiber	Distance from endplate to nearest fiber tip (% of total length)		No. of fibers measured	Fiber length (mm)	
	Mean	Range		Mean	Range
Intact	30		1	12.6	
One tip broken	36	28–48	9	9.9	6.1–13.1
Both tips broken	44	34–49	5	10.4	9.1–11.6

cation), although muscles of the ulnar group all exceed 5 cm in length. Adrian (3) found tenuissimus muscle fiber lengths of 13–23.5 mm with a mean of 17.3 mm for 21 intact fibers from a 12-cm long muscle; the longest fiber isolated from any muscle was 27 mm.

End-plates generally lie in the midportion of muscle fibers (5). The present results from electrical recording and by staining (Table I) agree by showing that end-plates are located predominantly in the middle third of intact fibers.

Summary. Single motor nerve fibers to bat web muscles were stimulated *in vivo,* and both ends of single muscle fibers were located by the presence of monophasic action potentials. The mean length of 12 such muscle fibers was 10.1 mm. Electrical responses of minimum latency were recorded in 6 of these fibers, demonstrating end-plate loci within the middle two quarters of muscle fibers, generally close to the middle. In addition, 71 muscle fibers were isolated after acid treatment. Mean length of 57 intact fibers was 14.1 mm and of 14 fibers with missing tips was 10.1 mm. The 14 fibers with missing tips and one intact fiber were stained for "lead-reactive substance" to locate their end-plates, which were within the middle two quarters of the fibers.

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Biliary Excretion of Phenol 3,6-Dibromphthalein Disulfonate in Rats Fed a Protein-Free Diet* (32802)

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It is generally accepted that much of the BSP (sulfobromophthalein sodium, phenoltetrabromphthalein disulfonate) taken up by liver cells undergoes conjugation with glutathione, and that conjugated BSP compounds account for most but not all of the dye excreted into bile of man, rats, and dogs (1–3). Whereas conjugation does not appear to affect hepatic uptake of BSP (4,5), evidence has been adduced that conjugation facilitates biliary excretion of the dye (4), and is an important determinant of the maximal rate at which BSP is transported from liver cells into bile (5). In the latter studies (5), feeding rats a protein-free diet for 2 days resulted in a decrease in the components of the hepatic BSP–glutathione conjugating system, with a 70% decrease in hepatic glutathione content, and a 25% fall in BSP–glutathione conjugating enzyme activity. When doses of BSP of 7.5 mg/100 gm of body weight and higher were administered intravenously to these animals, excretion of BSP into bile was diminished due to decreased excretion of conjugated dye. Biliary excretion of conjugated and total BSP was restored to control values, however, when hepatic glutathione content was maintained near normal levels by feeding a protein-free diet supplemented with 1% cystine.

The recent demonstration by Javitt (6), that phenol 3,6–dibromphthalein disulfonate, (diBSP) a compound related structurally to BSP, is readily excreted into bile of rats and

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