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The Leg Flexor Muscle of *Carcinus*. I. Innervation and Excitatory Neuromuscular Physiology

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ABSTRACT The innervation and neuromuscular physiology of the flexor muscles in the legs of *Carcinus* were investigated using a variety of histological and electrophysiological techniques. Slow muscle fibers were found in the proximal and distal regions of the muscle; intermediate- and fast-contracting fibers were found in most regions, but fast fibers predominated in the central regions. Muscle fibers were innervated by one to four excitatory axons. The extent of inhibitory innervation was not determined. The motor innervation showed significant departures from the previously described Brachyuran pattern. The results indicated the need for a thorough reexamination of the patterns of limb innervation of the crustacean tribes. *Key words* crab, innervation pattern, methylene blue, electron microscopy, electrophysiology, fiber type

The mechanism of control of muscular contraction is a central issue in the study of locomotion and posture in mammals and arthropods, because it is by means of this system that changes in the walking pattern or to posture are affected. Indeed, when investigating a tension receptor system (see Parsons, '80) the capabilities of the muscle that generates that tension must be understood if the proprioceptive qualities of the system are to be considered. Many workers have looked at innervations patterns, neuromuscular physiology, muscle fiber contraction types and proprioceptors in the simpler crustacean preparations which are usually innervated by no more than two excitatory axons (for reviews see Waterman, '61; Mill, '76; Hoyle, '77). Only a few workers have explored the more complex arthropod systems that show a greater diversity of muscle types and which are innervated by more than three excitatory axons (e.g., Phillips, '80).

Wiersma and Ripley ('52) have reviewed the leg innervation patterns of a variety of decapod crustaceans spanning four Reptantian tribes. By using methylene blue staining, single axon stimulation, and in some cases, silver-stained preparations of the motor nerves (van Harreveld, '39a), they reported that the excitatory innervation was remarkably uniform between the tribes whereas the inhibitory innervations were quite diverse.

The innervation of the legs of the Brachyuran *Carcinus* has not been studied, but most Brachyurans investigated have shown quintuple motor innervation in the leg flexor muscle of the carpus (Wiersma, '61).

In *Panulirus* (Tribe: Palinura), the leg flexor muscle has already been investigated in more detail (van Harreveld and Wiersma, '39; Furshpan, '55). The muscle is innervated by five motor axons. Two of the four excitatory axons produced fast contractions, and two gave slower contractions. Individual muscle fibers were innervated by from one to four excitatory axons, and the fifth and smallest axon, the inhibitor, apparently supplied all muscle fibers.

No published work on crustacean leg innervation patterns, in particular on the leg flexor muscles, has reported histological sectioning as a means of examining the innervation. This omission is hard to justify on two counts. First, the capricious nature of methylene blue as a means of staining nerve fibers has been known for a long time (Pantin, '64; van Harreveld, '39a,b). Second, very small nerve fibers may have electrical responses with amplitudes below the noise level of a recording system that uses extracellular electrodes. These problems have probably led to some erroneous conclusions concerning the innervation patterns.

The intrinsic contraction type of single muscle fibers within a given whole muscle can be revealed directly by intracellular depolariza-

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tion of single muscle fibers while monitoring the tension and the membrane response. Axonal stimulation of single motor units has been used but is unsatisfactory since motor units are known to innervate a range of intrinsic muscle fiber types (Jahromi and Atwood, '71; Atwood, '73). Sarcomere-length measurement, also widely used, is a poor measure of individual muscle fiber contraction type (Parsons, '82a).

The accessory flexor muscle (AFM), composed of a proximal and a distal head joined by a thin tendon, is also involved in controlling flexor contractions. In crayfish, this muscle has recently been shown to act as a "model reference system," whereby the flexor muscle is made to contract at the same speed as the AFM (Marrelli and Larimer, '79; J.L. Larimer, personal communication). The AFM in *Carcinus* will not be considered in any detail here.

Although examination of isolated muscular systems is in itself worthwhile, the information is much more valuable if it is related to the functioning of the whole, and preferably unrestrained, normal animal. Unfortunately, unambiguous recording of neural motor activity in specific leg segments in freely moving crabs is at present not technically feasible. The closest approximation to this ideal is to record from minimally restrained animals, and this type of preparation is used here.

In this paper, electron microscopic examination of the motor nerves is combined with the traditional methods of methylene blue staining and conventional electrophysiological techniques to examine the innervation and neuromuscular physiology of the flexor muscle of *Carcinus* walking legs. The distribution of the muscle fiber types as determined histochemically, and the physiology of detection of flexor muscle tension by the apodeme tension receptors is described elsewhere (Parsons, '82a,b).

MATERIALS AND METHODS

General methods and anatomical nomenclature are as described previously (Parsons, '80). To allow simultaneous recording of muscle membrane potential, flexor motor activity, and the tension developed by flexor muscle fibers in the whole animals, a minimally dissected and restrained preparation was utilized. The coxal levator muscle apodeme on the leg of interest was cut to prevent autotomy. Dental wax was used to secure the crab, with its carapace oriented vertically, in a perspex tray. To prevent the animal destroying the electrodes by moving its legs, all legs not being examin[ed] were anchored in wax proximal to the mer[us]-carpus (M-C) joint. All six walking legs w[ere] examined at various times. No substantial [dif]ferences were found between these legs. T[he] most distal pair of legs, which are modified [for] swimming, were not examined.

The required leg was fixed in position w[ith] wax and strong thread so that the segme[nt] distal to the merus remained free to move. C[uti]cle on the anterior face of the merus was [re]moved with a dental drill and the exten[sor] muscle was cut free to expose the flexor musc[les]. the accessory flexor muscle, and the main [flexor] nerve (MLN), which contains the flexor mo[tor] nerve (FMN) along its posterior edge. Bl[ood] loss from the exposed merus was controlled [by] gently packing the proximal end of the expo[sed] area with a large number of minute paper t[is]sue (Kleenex) balls and by ligaturing the ma[jor] blood vessel there with fine wire. A small w[ax] dam was built up around the merus to all[ow] chilled *Carcinus* saline (Parsons, '80) to co[ver] the muscle. The saline was replaced f[re]quently. Seawater was added to the tray to [a] level just below the dissected merus, so th[at] the crab was able to ventilate its gills un[as]sisted. If the water level was not high enou[gh] to allow proper ventilation, gas exchange w[as] assisted by pumping seawater into the o[pe]n opening of the gill chamber, using a peristal[tic] pump. The capacity to ventilate the gills [was] shown by water flowing outward from the [gill] region), and rapid withdrawal of the eyes [on] their sockets when touched, were taken as sig[ns] that the preparation was healthy. Bath te[m]perature was held at 12° to 14° C with [a] Peltier thermoelectric battery (Cambi[on] Thermionic Corp., Mass., U.S.A.).

Tension developed by flexor muscle fibers w[as] measured with a DMC Dual Mount Deflecti[on] Sensor (Kistler-Morse Corp., Wash., U.S.[A.]) mounted on a micromanipulator. The compl[i]ance of the system was 10 μm/gm. The tra[ns]ducer was attached to the carpus by a peg wh[ich] was inserted at one end into a hole in the ca[r]pus and held at the other by forceps fasten[ed] rigidly to the transducer. The transducer w[as] linear over the range of tensions encountere[d] here. The tension characteristics measured [for] comparison of muscle fiber contraction type w[ere] the time to 95% peak tension and the time fr[om] peak tension to 50% relaxation.

Conventional electrophysiological stimul[at]ing and recording techniques were used. Mo[st] opolar rather than bipolar silver hook el[ec]

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trodes were used because stimulus voltages could be greatly reduced and the stimulated nerve survived for a much longer period. The stimulating electrode was variously the anode or the cathode depending on which configuration provided excitation at the lowest level. Swapping the polarity sometimes resulted in selective stimulation of different axons in the nerve. The hooked nerve and electrode-wire shank were insulated from the bath by coating them with an adherent mixture of paraffin oil and vaseline applied through a syringe. This method allowed all muscle fibers to be in continuous contact with the chilled saline. Polyethylene-tipped suction electrodes were used to record from and to stimulate cut nerve endings. Glass capillary microelectrodes used for muscle recording and stimulation had resistance of 2–10 MΩ and were filled with a 3 M potassium chloride solution, but in some experiments a 2 M potassium citrate solution was used. Most muscle membrane penetrations were made with semifloating microelectrodes into the middle one-third of the muscle fiber, and deeper fibers were impaled by advancing the microelectrode either through the surface fibers or between muscle bundles. In some cases, muscle fiber membrane potential was monitored with a second glass microelectrode. Unless otherwise specified, all extracellular electrical stimulations were just suprathreshold.

Bursts of FMN activity that were elicited in the whole animal preparations were generated centrally by the animal. Excitation of flexor motoneurons occurred in response to several types of tactile stimulation to various parts of the body (see Results).

Isolated walking legs were obtained by inducing autotomy and were dissected and treated electrophysiologically as described for the whole animal preparations. In some cases, tension was measured directly from the disarticulated end of the flexor apodeme instead of from the carpus.

Histology

Physiological preparations were poststained with methylene blue to check electrode placement. Methods for methylene blue staining, electron microscopy, light microscopy, and cobalt backfilling have been described elsewhere (Parsons, '80). For the work described here, 1,100 mOsm of d-glucose was added to the cobalt acetate solution, because it was found to increase the distance moved by the cobalt and the percentage of successful fills.

RESULTS

Flexor muscle anatomy

The merus of the crab walking leg contains four muscles: the extensor, the flexor, and the distal and proximal heads of the accessory flexor (DAFM and PAFM). Carpus flexion is achieved primarily by contraction of the pinnate flexor muscle, and extension of the carpus occurs as a result of extensor muscle contraction.

All flexor muscle fibers insert on the flexor apodeme which is connected to the carpus by two flexible articulations. The first is the pivot at the flexor-apodeme to carpus junction itself, and the second is situated nearby just distal to the point where the DAFM apodeme joins with the flexor apodeme. Some of the most proximal flexor muscle fibers insert between these two articulations. These articulations probably allow the forces developed by the flexor muscle to be transmitted freely to the carpus throughout the whole range of angles of the M-C joint. Further details of flexor apodeme morphology are similar to those described for *Cancer* (Macmillan and Dando, '72).

The flexor muscle is composed of more than 200 individual fibers. A large proportion of the fibers appear to be loosely connected to their neighbors by fine tissue bridges. The criterion used to identify single fibers was that the fiber would easily separate from its neighbors when lightly grasped with forceps. When viewed from the anterior surface, the fibers are arranged in groups that are delineated from each other, first by a discernable change in the angle the group of fibers makes to the apodeme, and second, by the presence of small spaces between the fibers of adjacent groups. Branches of the main blood vessel often run through these spaces to supply the deeper (more posterior) areas of the flexor muscle. Nine of these muscle groups are present on the axial (dorsal) side of the apodeme, and three groups are found on the peripheral (ventral) side. For convenience, these groups are labeled A1 (distally) through to A9 and P1 to P3 respectively (Fig. 1). The deep small-diameter proximal muscle fibers (approximately below groups P3, A9, A8, A7) do not appear to be grouped this way.

Innervation pattern

Primary branching.

In methylene blue preparations, generally only the larger fibers in the FMN were stained. Electron micrographs of FMN sections, how-

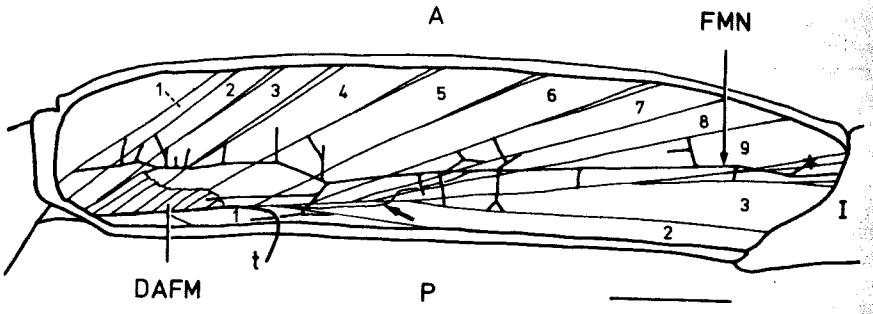

Fig. 1. Anterior view of the merus, showing the arrangement of muscle groups in the flexor muscle of *Carcinus* axial side; P, peripheral side. The primary lateral branches and the visible major secondary lateral branches of the fl motor nerve (FMN) in a typical methylene blue-stained preparation are also shown. The most proximal primary br (*) innervates the deeper fibers between muscle groups A9 and P3. DAFM, distal accessory flexor muscle; I, ischiu cut tendon of DAFM, which is folded back from the preparation. Arrow shows flexor apodeme sensory nerve. Scale ba 5 mm.

ever, showed that seven axons are present: four large-diameter fibers and three smaller-diameter fibers (Fig. 2a). The smallest fibers are sometimes just discernible in semithin sections stained with toluidine blue, but usually cannot be seen in paraffin wax sections examined with the light microscope. By varying the concentration, time, and temperature of methylene blue staining, it was sometimes possible to stain all seven FMN fibers simultaneously (Fig. 3). In some sections, more than seven axons were sometimes found. Subsequent selected sequential sections revealed that these extra axons were branches of one or more of the seven FMN axons.

When stained with methylene blue, the FMN is seen to branch regularly and extensively along the length of the flexor muscle (Fig. 1). The most proximal primary FMN branch forks posteriorly to innervate the deep proximal muscle fibers. Further distally, 11 primary lateral (i.e., dorsoventral) branches usually leave the FMN on the axial side of the apodeme. On the peripheral side six branches are usually present. These lateral branches then further divide to innervate nearby muscle fibers. Distal to the fifth primary branch on the peripheral side, the FMN separates from the MLN group to innervate the distal axial fibers. At this level, the most distal peripheral nerve branch arises to innervate the distal peripheral muscle fibers, and this branch divides further to supply motor fibers to the DAFM. Electron micrographs of the DAFM motor nerve show seven axons (Fig. 2b). This is in contrast to other Brachyurans in which only two axons are found supplying the DAFM from the FM (Govind et al., '78). Each of the primary FM branches contains at least the four thick e citatory axons; and many of the muscle grou (Fig. 1) are supplied by axons from more th one primary FMN branch. Each primary bran of the FMN divides further to innervate t individual muscle fibers throughout the flexo This finer branching naturally becomes ve complex and the details will not be dealt wi here. Due to the capriciousness of methyle blue staining, the extent of the innervation the three smaller FMN fibers that accompa the thick FMN axons was not determined this study. Cobalt fills of the FMN in situ co firmed the branching patterns shown by met ylene blue staining.

Some of the small axons that innervate t flexor muscle do not enter the merus with t FMN bundle. The axons branch from a para axon in the MLN at several levels along th merus. One, and sometimes two, of these fi fibers are seen in methylene blue-stained pre arations of the flexor muscle. These axons fro the MLN bundle run in parallel with the FM and its branches but are not connected lengt ways along the FMN. Each branch from th MLN fiber innervates only a discrete region the flexor muscle, although it runs within th FMN bundle as it does so (Fig. 4a). In meth ylene blue preparations where the extens muscle was left relatively intact, one of thes MLN fibers could be seen also to innervate th extensor muscle (Fig. 4b). This fiber appear to be continuous with the smallest of the thre extensor motor nerves that innervate the e

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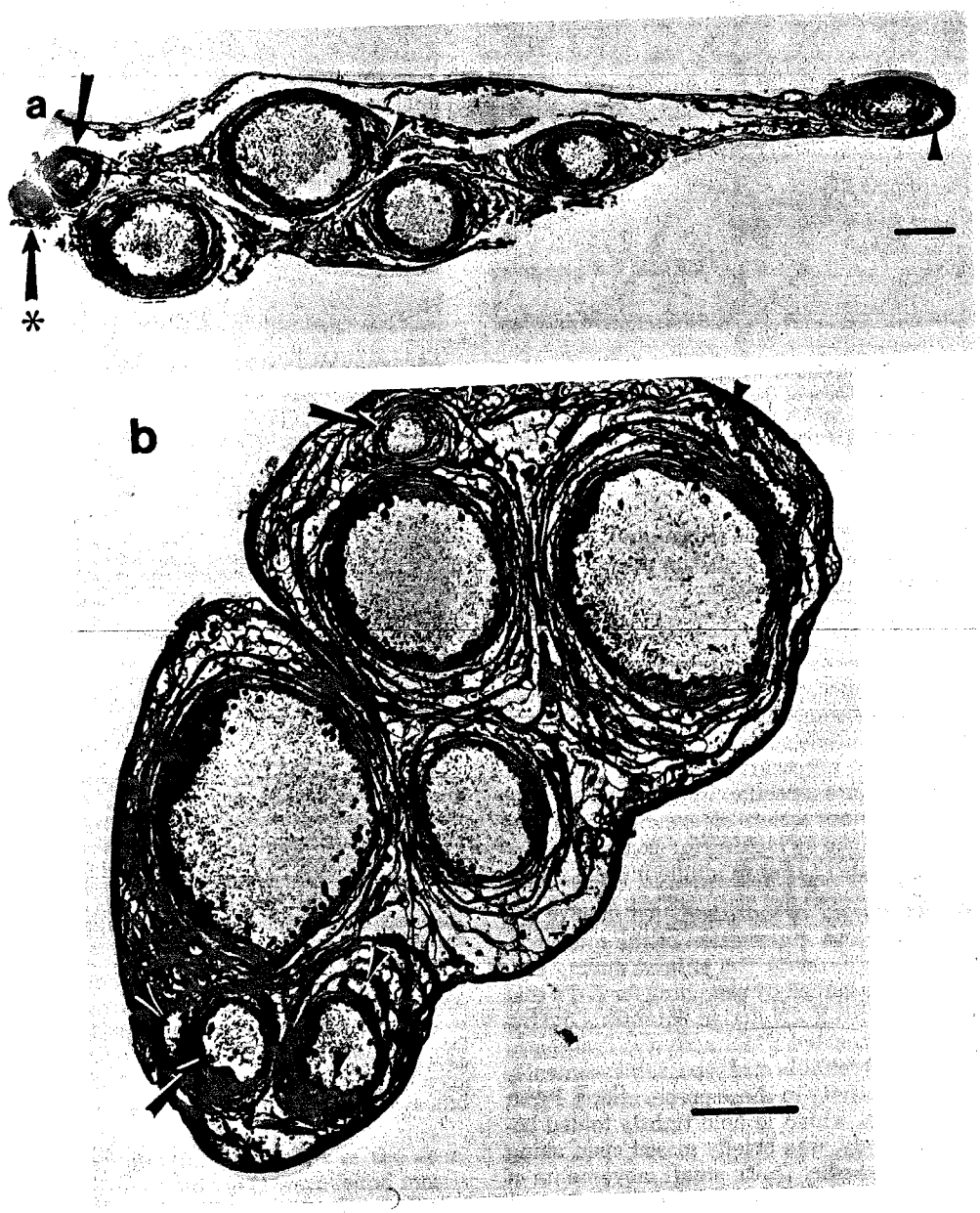

Fig. 2. Electron micrographs of the flexor muscle motor axon bundles. Long arrows indicate the two smallest-diameter axons. Arrowheads indicate glial cell nuclei. a. Flexor motor nerve distal to exit of the flexor apodome sensory nerve. Seven axons are present. The invaginated wall of the smallest-diameter axon (*) contains a glial cell nucleus. Note the similarity in the relative axon sizes with those in Figure 3. b. Motor nerve supplying the distal accessory flexor muscle (DAFM). Seven axons are present also. Scale bars: a, 5 μm; b, 5 μm.

tensor muscle. For ease of description, I have designated this fiber the common extensor-flexor (CEF) fiber. The parent axon in the MLN that gives off the CEF fiber runs through the merus into the distal segments. Its innervation in these distal segments has not been determined, but

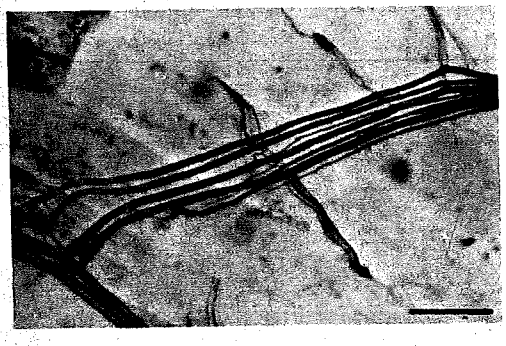

Fig. 3. Dorsal view of methylene blue preparation showing the seven fibers of the FMN, just distal to the sixth, peripheral side, primary FMN branch (Fig. 1). The distal end of the leg is to the right. Scale bar = 0.3 mm.

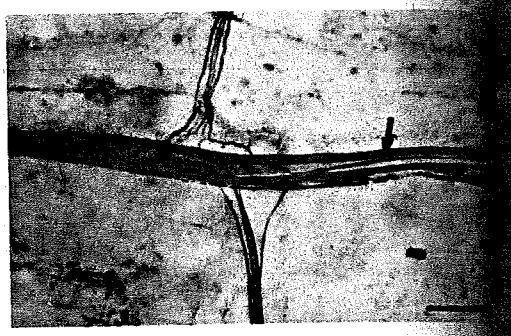

Fig. 4. The CEF fiber in the flexor muscle of *Carcin* Preparation stained with methylene blue. a. An example the noncontinuity of the common extensor-flexor (CEF) fi (arrow) along the length of the FMN bundle. This bran is at the third peripheral FMN branch point (see Fig CEF fiber arises from its parent axon more distally. So FMN fibers have lost their stain. Scale bar = 0.5 mm The branch of the CEF fiber that connects with the exte muscle is arrowed. This branch is found near the sixth mary peripheral branch of the FMN (see Fig. 1). This p tograph demonstrates a major problem of methylene bl staining. Most of the fibers of the flexor motor nerve have faded by the time that the finest fibers have taken the stain (compare with Fig. 3). Similarly, the fibers of th main leg nerve (bracketed) have also lost their stain. Sca bar = 0.3 mm.

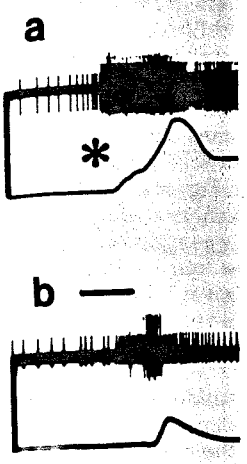

Fig. 5. Examples of cent tivity. Top trace, FMN activi ual abdominal flap stimula units are recruited for the bu and the tonic unit frequen Tonic unit frequency eventu els. The increase in tonic un burst of activity is due to the the abdominal flap. The ex units responding, discharge tion varies from animal to Stimulation here by brush joint cuticular membrane sometimes two phasic unit tonic unit discharge freque timulus levels. Scale bars: 0.5 gm.

when the CEF fiber was stimulated, action potentials were recorded in the opener muscle motor nerve in the propus.

Physiology.

An apparent anatomical connection between nerve and muscle does not necessarily mean physiological connection. In a polyneuronally innervated muscle such as the flexor, all motor fibers are not necessarily involved in all contractions. More specific information on the functional innervation of muscle fibers was therefore gained by looking for excitatory junction potentials (EJPs) in single muscle fibers.

Whole animal preparation. An idea of the distribution and innervation of the muscle fibers actually used by the animal during contractions was gained by searching for EJPs that correlated with FMN action potentials during centrally generated FMN activity. Two methods produced reliable and repeatable, centrally generated activity in these preparations. First, the abdomen, which is held tightly folded beneath the body, was briefly prised open using a mounted needle. Quite small movements of the most distal segment were usually sufficient to elicit a strong burst of short-latency activity in the FMN (Fig. 5a). Second, the cuticular membrane that spans the ventral face of the merus-carpus joint was gently stroked with a fine camel hair brush (Fig. 5b). This also elicited a burst of FMN activity, probably in response to afference from the sensitive chordotonal organs MC1 and MC2 situated at this joint (Mill, '76), but the response was less extended than that produced by abdominal flap stimulation.

In the resting animal, ongoing FMN activity usually consisted of one, and occasionally two spontaneous motor units that fired tonically at low frequencies (for example, see prestimulus activity in Figs. 5-7). Sometimes no motor activity was present until stimulation commenced. The frequency of the tonic unit increased slightly and briefly with mild mechanical stimulation such as taping the bench or carapace, and with manual manipulation of other legs or chelipeds. With the more vigorous stimulations described above, the tonic unit frequency increased or rarely three phasic a high frequency for these phasic units cor increases in flexor t with sudden movem chelipeds.

Excitatory junctio taneously active ton in the more posterior distal ends of the fle the tonic unit EJPs whereas in others, t until facilitated by t quency during moto 6a,b).

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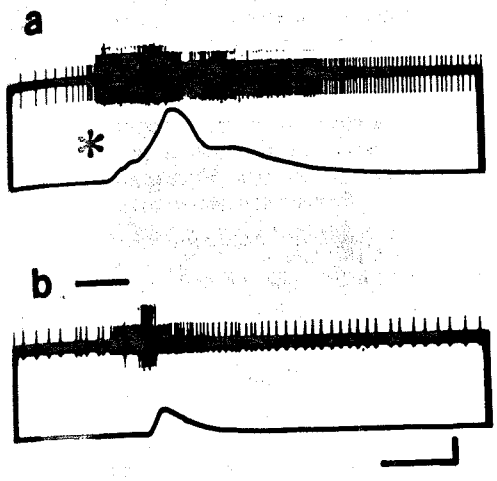

Fig. 5. Examples of centrally produced flexor motor activity. Top trace, FMN activity; lower trace, tension. a. Manual abdominal flap stimulation (at *). At least two phasic units are recruited for the burst of FMN activity that ensues, and the tonic unit frequency also increases considerably. Tonic unit frequency eventually returns to prestimulus levels. The increase in tonic unit activity just prior to the main burst of activity is due to the positioning of the probe behind the abdominal flap. The extent of the response (number of units responding, discharge frequency, and response duration) varies from animal to animal (e.g., see Fig. 6a,b). b. Stimulation here by brushing of the merus-carpus (M-C) joint cuticular membrane (upper bar). Usually one, and sometimes two phasic units are recruited. The increased tonic unit discharge frequency eventually returns to prestimulus levels. Scale bars: a, 1 second, 4 gm; b, 1 second, 0.5 gm.

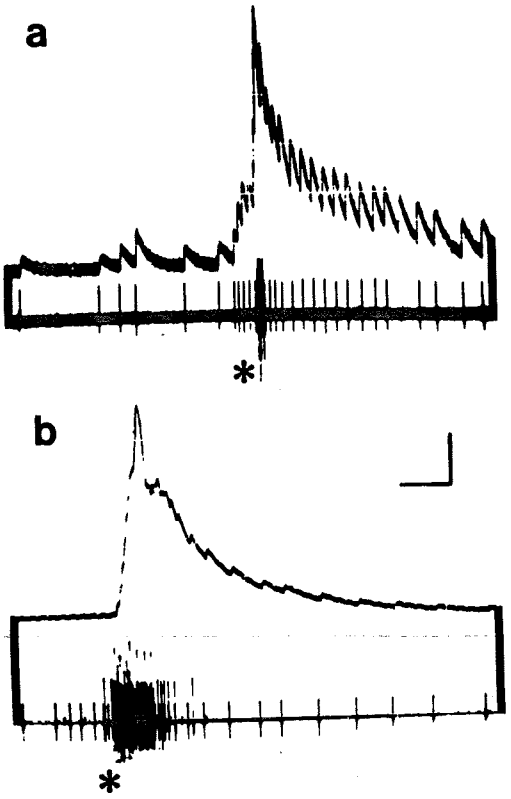

Fig. 6. Membrane-potential responses of muscle fibers to activity in the FMN following abdominal flap stimulation (*). Top trace, muscle fiber membrane potential; lower trace, FMN activity. a. Microelectrode in muscle group, A6, Second fiber down. In this fiber, tonic unit excitatory junction potentials (EJPs) are apparent at all times and show facilitation during response. b. Another preparation, microelectrode in muscle group A4, third fiber down. Here, tonic unit EJPs are not evident until the response occurs, and the amplitude dies off rapidly after the burst of FMN activity. The smaller of the phasic units does not innervate this fiber. Whether the larger phasic unit innervates this fiber cannot be determined here. Scale bars: a, 0.1 second, 5 mV; b, 0.1 second, 10 mV.

unit frequency increased rapidly and one, two, or rarely three phasic units were recruited at a high frequency for a short period. Bursts in these phasic units correlated with sudden large increases in flexor tension (Fig. 5a) and also with sudden movements in the other legs and chelipeds.

Excitatory junction potentials of the spontaneously active tonic unit were found mostly in the more posterior fibers at the proximal and distal ends of the flexor muscle. In some fibers the tonic unit EJPs could be seen at all times, whereas in others, the EJPs were not evident until facilitated by the increased tonic unit frequency during motor unit burst activity (Fig. 6a,b).

Phasic unit EJPs were found widely distributed throughout the flexor muscle fibers. Often one phasic unit and the tonic unit innervated these fibers (Fig. 7). More rarely, a second phasic unit was also present. EJPs in some fibers showed rapid facilitation, and muscle potential spikes were elicited in many muscle fibers if the spike threshold was reached. Other fibers could not be induced to spike—the potential merely reached a plateau of depolarization. In these whole animal experiments there were no clear examples of muscle innervation by four (or more) excitatory axons. This may have been due to spike superposition during the centrally generated, high-frequency bursts of FMN activity that were elicited here. As a result of

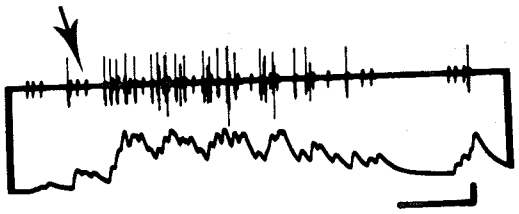

Fig. 7. Portion of the response of the FMN (top trace) and a muscle fiber membrane potential (lower trace) to abdominal flap stimulation. Correlation between the EJPs of the tonic unit (arrowed) and the next-largest, phasic unit is clear. Muscle fiber is located on group A6, three fibers down. Scale bar: 0.1 second, 10 mV.

this superposition, unambiguous identification of more than three motor units (and their EJPs in muscle fibers) was generally not possible. In most FMN responses only three flexor motor units were involved.

Isolated-leg preparations. The main features of the distribution of the excitatory motor axon in the muscle were examined by sampling muscle fiber EJPs during axonal stimulation of single FMN fibers in isolated preparations (e.g., see Furshpan, '55). A complete mapping of the innervation of fibers in this muscle was not possible because of the large number of fibers that would have to be sampled, because of the large number of axons, and because some of the excitatory axons fatigue rapidly. Instead, the muscle fibers sampled in each preparation were grouped into three broad areas: proximal, central, and distal. A similar approach has been used in other crustacean muscle innervation studies (e.g., Govind and Lang, '74).

Due to the small diameter of the fibers, it was not possible to physically separate the seven individual axons of the FMN, but commonly three and sometimes four of the thick excitatory axons were isolated for stimulation. Activity in the FMN was monitored distally as a check on the number of fibers stimulated. By combining the results of these EJP investigations with visual observations of the areas of fibers that contract following stimulation, an idea of the extent of individual motor unit innervation was gained. At least one of the units that elicits rapid flexor contraction apparently innervates all fibers in the anterior levels of the muscle. One slow unit innervates a large number of the more posterior proximal muscle fibers and also fibers of the most di fiber groups, but scattered fibers elsewhere the flexor are also innervated by this unit. T innervation of the two remaining excita axons present in this muscle is not as cle however, as both these axons appear to inn vate some fibers in all muscle groups. In flexor muscle of *Panulirus*, Furshpan ('55) fou that very few fibers were innervated by all f excitatory axons. Since it was not possible h to routinely stimulate the four excitatory ons individually (see above), the extent quadruple excitatory innervation in the fle of *Carcinus* could not be determined.

These isolated-leg preparations yield inf mation on the range of muscle responses a on the innervation of particular fibers. Unfo tunately, the identity of the excitatory axo cannot be determined directly because the di charge and recruitment properties of the co responding motor neurons are absent. Ho ever, if the results from the isolated-leg a whole animal preparations are combined, t conclusions can be made: first, that the ax which innervated predominantly the de proximal and distal muscle fibers in the is lated leg preparations, and which elicited slo tensions in the fibers it innervated, is one the tonic units; and second, that the axons th innervate most of the muscle fibers and th elicit rapid large-tension increases are phas in nature.

Intrinsic muscle fiber contraction types

To reveal the intrinsic contraction propertie of muscle fiber, the fiber was directly depola ized with current introduced through a mi croelectrode, and at the same time the mem brane response and the tension produced were monitored. The range of possible muscle-fiber responses to direct (intracellular) stimulation has already been described (*Carcinus* closer muscle, Atwood, '63) and will not be repeated here. The flexor muscle fibers examined here show similar responses to those seen in the closer muscle, but when the flexor fibers are considered in broad fiber-type groups (fast, intermediate, and slow), differences in the distribution of these fiber types become apparent. Fast-contracting fibers that showed spike responses (Fig. 8a) were located at all levels along the flexor, predominantly in the more central and anterior levels. Some of these fibers spiked repeatedly if the depolarization was maintained (Fig. 8b). Intermediate fibers, i.e., those with speeds of contraction and/or relaxation intermediate between the fast and slow fibers

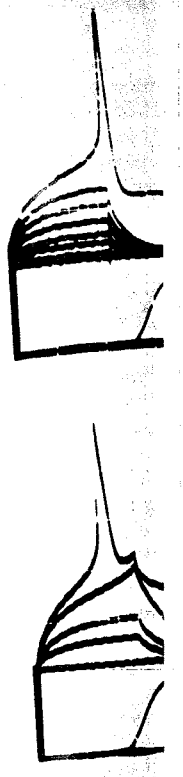

Fig. 8. Respo membrane poten muscle fiber, sh occurs. Fiber in Fiber in group A 500 msec. c. An relaxation than fibers down. d. many slow fiber 200 msec. Scale

described h flexor (Fig. graded or s fibers were of the prox distal ends response o tracellula response (at high de time-cour delayed r ization (e For a g showed ited twit

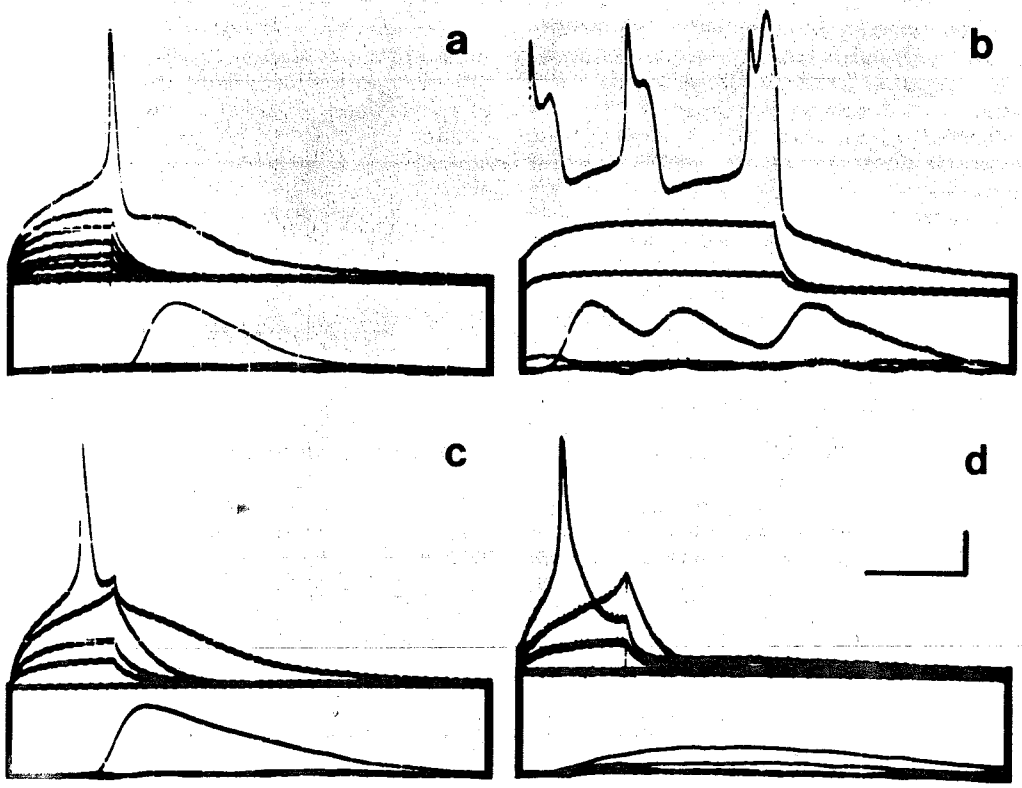

Fig. 8. Responses of selected muscle fibers to increasing voltage single-pulse intracellular depolarizations. Top trace: membrane potentials. Lower traces: tension, measured at the carpus. All responses are from one preparation. a. Fast muscle fiber, showing action potential in response to depolarization. No tension is developed until the action potential occurs. Fiber in group A5, second fiber down. b. Repeated spiking in a fast muscle fiber due to maintained depolarization. Fiber in group A6, second fiber down. The reason for the double-peaked spikes is not known. Stimulation for approximately 500 msec. c. An intermediate type muscle fiber, showing an action potential and a similar contraction rate, but with slower relaxation than the fast fiber shown in a. Other fibers also have slower contraction rates. This fiber is in group A5, three fibers down. d. Slow fiber, showing graded response and a slow spike to depolarization. Fiber located deep in group A8. In many slow fibers, the tension developed is barely discernable. In a, c, and d, the applied depolarization is for approximately 200 msec. Scale bar: 0.2 seconds; tension, 0.04 gm; muscle potential, 9 mV.

described here, were found at all levels of the flexor (Fig. 8c). These fibers usually showed graded or spike responses. The slowest muscle fibers were present in the more posterior parts of the proximal, and to a lesser extent, of the distal ends of the flexor muscle. The membrane response of these slow-contracting fibers to intracellular depolarizations was either a graded response (sometimes with slow spike responses at high depolarization levels—Fig. 8d) or a slow time-course electronic potential that showed delayed rectification at high levels of depolarization (e.g., see Atwood, '65; Fig. 1).

For a given stimulus duration, the fibers that showed spiking membrane potentials exhibited twitchlike fast contractions and produced the greatest tensions. However, when fibers in the most anterior layer of the flexor were depolarized, twitch-type contractions slower than those of the fast fibers were observed and spiking muscle membrane potentials were present, but often little or no tension was produced.

Muscle fibers adjacent to those undergoing intracellular depolarization were checked for any depolarization of their own membrane potential. This was necessary to ensure that the contractions were not spreading to other muscle fibers. Only at excessively high stimulus voltages, considerably greater than threshold values for a fiber under study, was any leakage depolarization encountered. These high voltages were not used.

Fatigue resistance is a property of slow muscle, and although there are exceptions (e.g., Silverman and Charlton, '80), it is poorly developed in fast muscle. The fatigue resistance of the various fast, slow, and intermediate single muscle fibers was briefly examined using repeated intracellular stimulation. Although spontaneous fast and slow contractions in other fibers (which are also recorded by the system used here to measure tension) made recording of long-term slow muscle stimulation trials impossible, the overall picture was clear: slow fibers (identified by their intrinsic contraction profile) fatigued slowly, while fast (e.g., Fig. 9) and intermediate fibers fatigued more quickly.

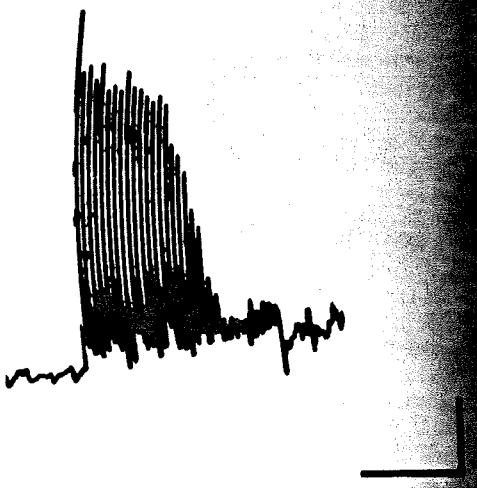

Fig. 9. Tension response (measured at the carpus) to ... peated (0.2 Hz) intracellular depolarization (80 V, 80 m... of a fast contracting fiber in group A4, several fibers do... The contractions fatigue rapidly after an initial peak in ... tensions. Changes in baseline tension are due to spon... neous contractions in other flexor fibers. Scale bars: 30 s... 0.01 gram.

DISCUSSION

The results presented show that the flexor muscle in the merus of the walking legs is a heterogeneous muscle that contains a range of intrinsic muscle fiber contraction types, from extremely fast through to the extremely slow. Intrinsically slow-contracting fibers are localized in the proximal and distal portions of the muscle, and faster contracting fibers are present throughout the muscle. Seven axons are available to innervate the muscle fibers.

In a typical section of the FMN (Fig. 2a) the ratio of the diameters of the smallest to the largest nerve fibers is approximately 1:26. In contrast, the corresponding ratio in *Panulirus* (van Harreveld and Wiersma, '39; Furshpan, '55) averages approximately 1:4. Furthermore, the smallest fiber in *Panulirus*, the inhibitor, is approximately the same size as the largest fiber of the *Carcinus* flexor motor nerve. These size differences mean that while apparently all fibers in *Panulirus* can be easily observed and stimulated, methylene blue staining or individual stimulation of the smaller fibers in the FMN of *Carcinus* is usually impossible. Since it was already apparent in the early crustacean innervation studies that methylene blue staining of small fibers was unreliable (van Harreveld, '39a) it is strange that no mention of confirmation of fiber numbers, by routine histological or electron microscopical methods has been made in the literature.

In *Carcinus* the presence of an extra two fine fibers in the FMN was not apparent until tissue fixed for electron microscopy was examined. It is tempting to speculate that other small fibers may be present in crustacean motor axon bundles that have been examined without the use of electron microscopy, since the electrophysiological methods used to examine innervation by the smallest fibers (inhibitory ?) might overlook very small fibers and are unlikely to distinguish between simultaneous stimulation of one or of several inhibitory axons. A rei... vestigation of the innervation of some inse... muscles (Walther, '80) has shown the presenc... of small-diameter fibers that had been ove... looked in the original investigations.

The results show clearly that the innerva... tion patterns of the flexor and accessory flexo... muscles in this crab do not agree with th... Brachyuran scheme presented by Wiersma ('61... Electron micrographs (and one methylene blu... preparation) have shown two additional sma... fibers present in the FMN (Figs. 2a, 3), in ad... dition to the accepted Brachyuran scheme wit... its four thick (excitatory) axons, and one thi... (inhibitory) fiber. The DAFM is innervated her... by seven axons, in contrast to an innervation... of one excitor and one inhibitor reported in thi... muscle for all crustaceans that have been ex... amined so far (Wiersma, '61; Govind et al., '78). In crustacean motor fiber bundles, and in par... ticular in the muscles of the merus, excitatory fibers usually have larger-diameter axons than inhibitory fibers (Furshpan, '55; van Harrev... eld, '39b). Stimulation of each of the four large *Carcinus* FMN axons did elicit muscle contractions, confirming that the four larger FMN fibers were excitatory.

It has not been determined here whether all the remaining three small fibers are inhibitory, but at least one FMN fiber will be inhib... itory according to the accepted innervation scheme for the Brachyura. This is the "common

inhibitor," which innervates both the opener muscle and the flexor muscle. Since the CEF in *Carcinus* is physiologically linked to the opener muscle motor nerve, it is reasonable to assume here that the CEF fiber is the common inhibitor of the flexor and opener muscles. The CEF fiber also supplies the extensor muscle and is apparently continuous with the third, smallest-diameter axon of the extensor motor nerve. This finding also supports the notion that the CEF fiber is an inhibitor, since the extensor inhibitor is normally the smallest-diameter fiber innervating this muscle. This common inhibitory innervation between the limb flexor and extensor muscles is present in Anomuran and Palinuran decapods (Mill, '76; Hill and Lang, '79), and has also recently been demonstrated physiologically in *Cherax*, an Astacuran (Cooke and Macmillan, personal communication). Taken together, these findings suggest that the inhibitory innervation patterns in the decapoda may not be as diverse as previously thought.

Whether any flexor muscle fibers here receive all seven axons is not known since no attempt was made to examine the inhibitory axon physiology. The results do show that the flexor muscle fibers were innervated by up to four excitatory axons. The majority of the flexor muscle fibers were supplied by the first phasic axon that is recruited in centrally generated flexor motor nerve bursts of activity. (Fig. 5a). The spontaneously active tonic motor unit innervates many muscle fibers in the posterior proximal and the distal muscle fiber groups of the flexor muscle, which correlates with the presence of intrinsically slow muscle fibers situated there. Some fibers were innervated by both these axons, and smaller number of fibers were supplied by three axons. It is clear then, that muscle fibers are not necessarily innervated by all axons, and this finding is consistent with results from other complex arthropod muscles (Furshpan, '55; Phillips, '80).

If the flexor muscle is viewed as an assemblage of relatively discrete muscle fiber groups (Fig. 1) it is possible to make useful generalizations on the reasons for the innervation pattern of these groups, and on the control of flexor contraction as a whole.

The slowest flexor contractions involve muscle fibers in the deep proximal layers, and to a lesser extent, in some of the most distal fibers in the merus. The fastest contractions are found in muscle fibers toward the center of the merus, and predominantly in the superficial (anterior) half of the muscle. Within most of the muscle groups (Fig. 1) muscle fibers are found that produce intermediate speeds of contraction. For a given length stimulus, the largest tensions are produced by contraction of the more centrally placed muscle fibers while smaller tensions are developed by muscle fibers situated proximally or distally in the merus. Part of the reason for the small tension development (measured at the carpus) of the distal slow muscle fibers may be due to their large angle of insertion onto the apodeme compared to the more proximal muscle fibers. In the muscles of the propus of *Chionectes*, Atwood ('65) has shown that the large-diameter ("thick") fibers produce the greatest individual tensions, while the smallest tensions (for a given depolarization duration) are developed by the small-diameter ("thin") fibers. In general this relationship was also found here, and an analysis of fiber area in the flexor muscle of *Carcinus* (Parsons, '82a) shows that the large fibers predominate in the central regions, while most of the smaller fibers are found in the deeper areas at the proximal and distal ends of the muscle.

The functional significance of the localization of slow fibers at the proximal and distal end of the flexor is likely to be related to mechanical factors involved in the application of muscle fiber contractile forces to the carpus. For example, when the M-C joint is near the fully flexed position, the proximal slow fibers are flaccid and can transmit little or no force to the carpus. On the other hand, the distal slow fibers, which are at close to resting length in this situation, can transmit their contractile forces more efficiently through the apodeme onto the carpus. In this way, low-level postural adjustments at low M-C joint angles, mediated by activity in the tonic axon, could be effected. At larger M-C joint angles, slow low-level postural adjustments would be best accomplished by the slow fibers located proximally, since the losses in the transfer of their contractile forces to the apodeme (due to the lateral (= dorsal-ventral) component of their force vectors) are minimal here.

The whole animal experiments indicate that postural control of the flexor muscle in the resting animal can be regulated as in other crustacean systems (e.g., Cannone and Bush, '80) by the activity of the continuously active tonic unit. In these preparations the discharge frequency of the tonic unit at the resting tension level increases during mild mechanical stimulation and returns to resting levels when the stimulus is removed. These low-level adjustments mainly involve the deep (posterior) proximal slow muscles of the flexor. Rapid control of posture and/or locomotion, in response to more vigorous or noxious stimuli, can be achieved here by recruitment of phasic units

that fire briefly at high frequency to contract the faster muscle fibers and so elicit rapid and strong flexor muscle contraction. In most cases two phasic units were recruited, and often, just before the bursts of phasic unit activity, the tonic unit discharge frequency increased and was held at a higher level until the end of the response. Following particularly vigorous flexor contractions in these preparations, activity in the FMN was often inhibited for a short period, after which the tonic background FMN unit resumes firing. The mechanisms of this post burst inhibition are not clear, but may be associated with inhibition that can be produced by activity in the flexor apodeme sensory nerve (Parsons, '82b).

When stimulated intracellularly, many of the large, most superficial (anterior) fibers of the flexor muscle produced both membrane potential spikes and visual twitch contractions, but often developed little or no measurable tension at the carpus. Anomalies in the functioning of the most superficial muscle fibers of a muscle have been noted in another crustacean muscle. In *Cambarus*, the most superficial layers in the leg and claw muscles would not contract with any type of axonal stimulation, and it was thought these fibers were especially sensitive to mechanical damage during dissection (van Harreveld, '39a,b). The reason for the poor tension development in the superficial fibers here is not clear, but it may be associated with an interaction between fiber position, apodeme structure, and the method of tension measurement (Parsons, '82a).

The speed and strength of tension development in these muscle fibers clearly covers a wide range. It is likely that the spatial distribution of the fibers about the apodeme will also influence the way in which tension is detected by the tension-receptors, and the way it is transferred to the carpus.

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The Leg Flexor M...
Fiber Types

ABSTRACT Th... cle of *Carcinus ma*... tive enzyme NADH... of these fiber types... cross-sectional area... physiologically. *Ke*... oxidative capacity...

Investigation of the co... tacean muscle fibers has ... muscle fiber types. Two ... ily recognized: "slow" a... ate types can also be id... although they are bett... senting a continuum fro... other.

A variety of histologi... been used to identify mu... include histochemical ... triphosphatase (ATPas... zymes, and measure... length.

Traditionally, sarcom... used as an indicator of ... '76). Recently sarcom... fiber type have come ... onomous, with short ... equated to fast fibers ... bers being equated to ... '80; Ogonowski et al., ... may not be valid in all...

More recently, Lan... ATPase activity as w... to classify fibers. So... have arisen with atte... morphological and ... with physiological re... by the perplexing co... tive capacity of the ... rectly correlated with ... on adenosine triphos... comere length)" (Lar... it would appear to b... late metabolic status ... function. Close exam... graphs of Lang et al...

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