ELECTRICAL AND MECHANICAL CHARACTERISTICS OF A VERY FAST LOBSTER MUSCLE

MARTIN MENDELSON

From the Department of Physiology and Biophysics, New York University School of Medicine, New York 10016, and The Marine Biological Laboratory, Woods Hole, Massachusetts 02543

ABSTRACT

The remotor muscle of the second antenna of the American lobster is functionally divided into two parts. One part produces slow, powerful contractions and is used for postural control. The other part produces very brief twitches, can follow frequencies over 100/sec without fusion and is probably used for sound production. This great speed is due, in part, to synchronous arrival of nerve impulses at multiple terminals, a very brief membrane electrical response and electrical continuity throughout large volumes of sarcoplasm. Calculations indicate that the very extensive sarcoplasmic reticulum is probably responsible for the rapid decline of tension in this muscle.

INTRODUCTION

The lobster, *Homarus americanus*, is known to produce a buzzing sound when disturbed. Moulton (14) said of the vocalization of *Homarus*, "... vibration is at times so intense that an inexperienced person may drop the animal." Fish (7) employed a hydrophone to measure the underwater sound output of lobsters and found that they emit fundamental frequencies of 100–125 Hz. The fundamental is accompanied by a discrete series of harmonics, and the clear difference from the wide band grating noise produced by stridulating species led Fish to propose that the sound is produced directly by contraction of muscles at the base of the second antenna. The largest muscle in that position is the remotor muscle (22) of the second antenna. This report describes some of the mechanical and electrical characteristics of this muscle and shows that, like the sonic muscles of some species of fishes (4, 8, 23), it is capable of repeated contraction at frequencies adequate to account for the sound output of a lobster. The ultrastructure of this muscle has been described in the accompanying paper by Rosenbluth (20), and a preliminary report on its physiology has appeared previously (12).

METHODS

Lobsters were obtained from commerical dealers and kept in refrigerated, running sea water until used. For the recording of the electromyogram (EMG) of the remotor muscle in the intact animal, two holes were drilled through the carapace over the remotor and a 25 μ Pt-Ir wire, insulated to the tip, was inserted 1.5 mm into each hole. The two wires were then fixed in place with dental cement. After 5 days the wires were still in place and the animal was quite healthy. The potential difference between the two electrodes was recorded while the lobster was stimulated by prodding on the head.

For experiments on isolated muscles, the animals were opened by removal of the dorsal part of the carapace and the viscera were shaken out. The remotor of the coxa of the antenna is a large, prominent muscle mass which has its origin posterolateral to the

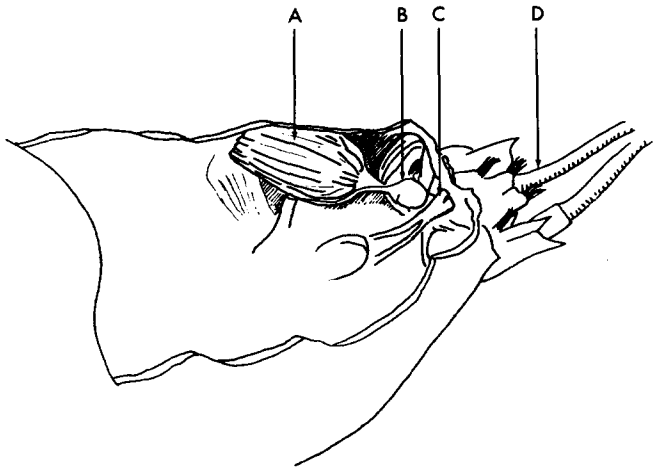

FIGURE 1 Location of the remotor of the coxopodite of the left second antenna. View from above of a lobster with the dorsal carapace removed. The muscle (*A*) is innervated by a branch of the antennal nerve (*B*) which arises from the supraesophageal ganglion (*C*). The flagellum of the left antenna is visible at *D*. Only the fast division of the muscle is visible.

base of the antenna as can be seen in Fig. 1, a diagram of the preparation at this stage. The muscle is served by a branch of the antennal motor nerve from the supraesophageal ganglion. When necessary the entire length of the nerve was freed from connective tissue and, after the branches to the other antennal muscles were cut, was removed with the remotor and overlying skeleton. The preparation was pinned down in a paraffin chamber, in a saline solution whose composition is given in Table I, and was cleaned of blood vessels and connective tissue. Either the entire nerve trunk, or a single fiber dissected out of it, was drawn into a suction electrode which could be used either to record or to stimulate. Intracellular potential records from the muscle fibers were made with KCl-filled micropipette electrodes and conventional display equipment. When desired, a second micropipette electrode could be inserted either to record potential or to pass current. In the latter instance, the tip of the electrode was usually broken to give a resistance of 0.5–1 MΩ.

The tension produced by the whole muscle was recorded by freeing the apodeme from the coxa and connecting it with a length of Dacron thread to a tension transducer, either a Grass FTO-3C strain gauge or an RCA 5734 isometric tension transducer.

In experiments where the Na^+ concentration was altered, NaCl was replaced with choline-Cl (twice recrystallized). When the Ca^{++} concentration was varied, the osmotic difference was compensated for by altered NaCl. All experiments were performed with the preparation at 16–17°C, except for recording of EMG's at 12.5°C. The innervation pattern was examined with methylene blue staining.

RESULTS

The remotor muscle of the second antenna is composed of two anatomically distinct parts (20). The major portion of the muscle complex consists of what appear to be large, coarse fiber bundles. As will be shown below, this portion is the fast division of the muscle. A small number of thinner, shorter fibers insert on the same apodeme, somewhat obliquely to the others. Their properties will be dealt with after those of the fast units have been described.

TABLE I

Physiological Saline for Homarus

Salt	g/liter
NaCl	26.39
KCl	0.39
$MgSO_4$	2.42
Na_2SO_4	0.81
$CaCl_2$	4.56
Dextrose	1.0
H_3BO_3 0.5 M	17.57 ml/liter
NaOH 0.5 M	0.956 ml/liter

Electrical Properties of Fast Division

The responses of fast division units to depolarizing transmembrane currents are summarized in Fig. 2. In this experiment, two microelectrodes were inserted about 250 μ apart on the longitudinal axis into a surface unit and outward current pulses of increasing magnitude were applied through one electrode while the other electrode monitored the resultant potential changes. In Fig. 2 A the potential change has the form of a passive charging of the membrane capacitance (hyperpolarizing currents produce results like those in Fig. 2 A, only

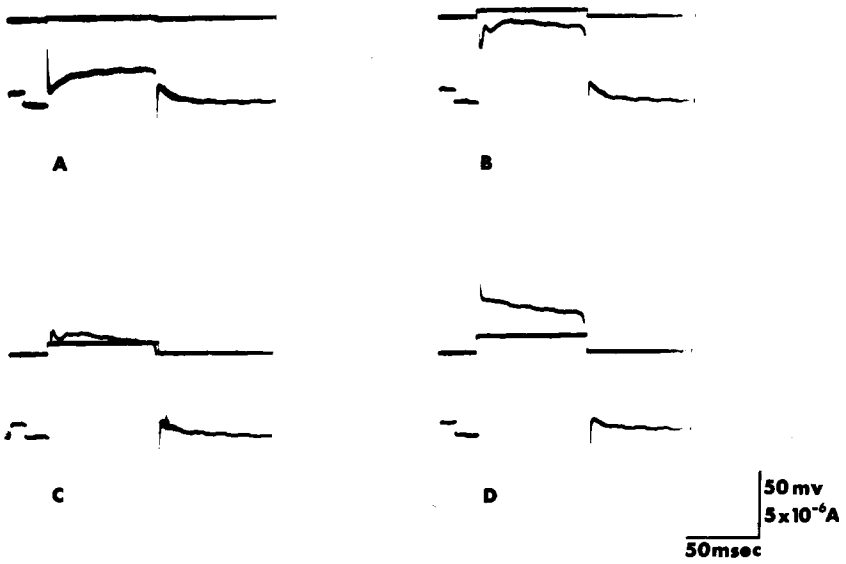

FIGURE 2 Responses of a surface unit of the fast division of the remotor. The upper trace of each part shows the current injected into the cell; the lower trace displays the consequent voltage change. Note the small electrically excitable response on the rising phase in *B* and *C*.

inverted and larger). In Fig. 2 B and 2 C small, transient depolarizing responses appear on the rising phases of the potential records. These are the electrically excitable response of the membrane to depolarization. Presumably, they are due to a transient increase in permeability to some ion whose equilibrium potential lies at a more positive level than that attained by the transmembrane potential. If this is true, then current-induced depolarization close to that equilibrium potential should lessen or abolish the response; Fig. 2 D shows that this does indeed occur: a large current-induced depolarization abolishes or obscures the response. In no instance was it possible to elicit a regenerative, all-or-none action potential by stimulating through an intracellular electrode with the muscle in normal saline: whenever active responses were observed they were graded with the magnitude of the stimulus. Graded electrical responsiveness of this kind is common in arthropod muscle (5, 6), and Werman and Grundfest (27) showed that gradation is caused by rapid repolarizing electrogenesis, probably an increase in K^+ conductance, triggered by the depolarization. This repolarizing electrogenesis and its dependence on membrane potential can be shown readily as in Fig. 3. In Fig. 3 B the lower trace shows the response of a fast division unit to stimulation of its motor nerve bundle. The initial depolarization is followed by a fast repolarization toward resting potential and then tails off into the end of the junctional potential. Fig. 3 A is composed of two superimposed records. In one the motor nerve bundle was stimulated and a pulse of hyperpolarizing current, monitored on the uppermost trace, was passed across the membrane during the rising phase of the response, so that the net depolarization was less than in Fig. 3 B. The superimposed trace in Fig. 3 A was produced by a hyperpolarizing pulse alone, and permits correction of the first response for the capacitive effects of the hyperpolarizing pulse. When the response is so corrected, it is found that the repolarization after the peak is slowed and that the response is prolonged as indicated by the dotted curve in Fig. 3 B. (The time relation of the hyperpolarizing pulse is indicated by the dashed line on the uppermost trace of Fig. 3 B.)

This same effect is seen with long current pulses alone. The steady-state increase in membrane conductance upon depolarization is shown in Fig. 4. The half-filled circles indicate the change from resting potential in fast units attained during the passage of long current pulses, either inward or outward. The slope of the curve at any point is $E/I = R_{eff}$, the effective resistance of the membrane. In the hyperpolarizing direction the points lie along a line with slope = 5 kΩ whereas in the

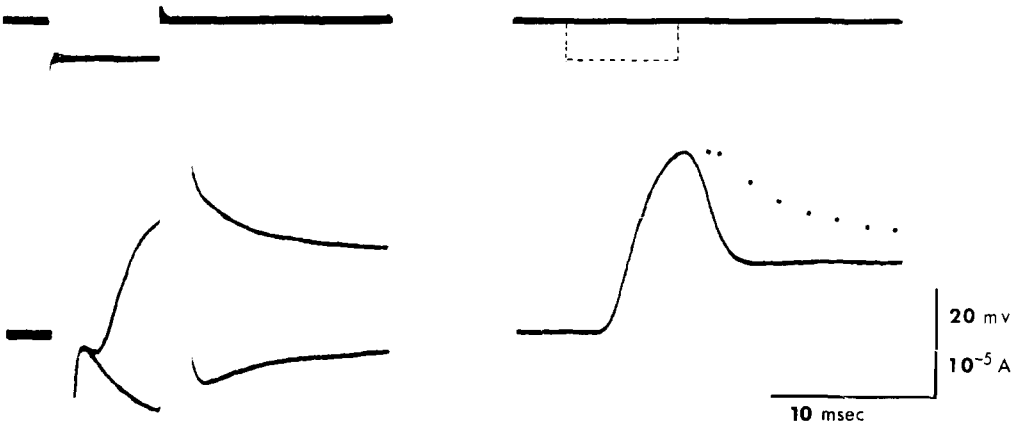

FIGURE 3 Dependence of repolarization rate on degree of preceding depolarization in the fast division. In part A, two sweeps are superimposed (the lower trace records transmembrane potential, the upper monitors hyperpolarizing current injected through a second microelectrode): in the first sweep a hyperpolarizing current pulse is imposed on the rising phase of excitatory junctional potential (ejp); in the second sweep only the current pulse occurs. Part *B* shows an uninterrupted ejp in the same unit (full line) and the effect on the time course of the falling phase (dotted curve) of the hyperpolarizing pulse, after correction for capacitive distortion. The dashed line on the upper trace indicates the time relation of hyperpolarization to ejp.

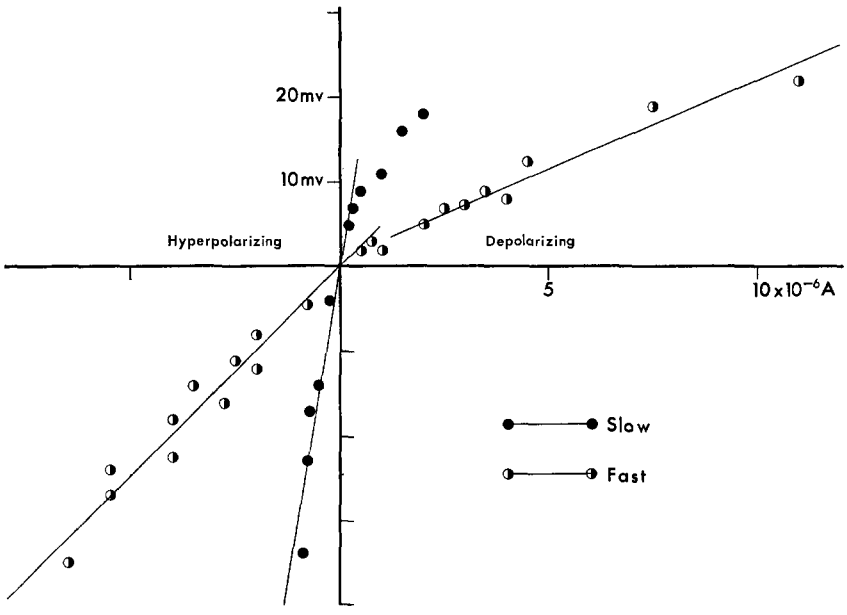

FIGURE 4 Current-voltage relation of representative units of fast (half-filled circles) and slow (filled circles) units of the remotor. The origin is at resting potential; depolarization of the unit is plotted upward and hyperpolarization downward. The lines indicate effective input resistance of: 5 kΩ for hyperpolarization of fast division, 2.5 kΩ for depolarization of fast division, and 100 kΩ for slow division.

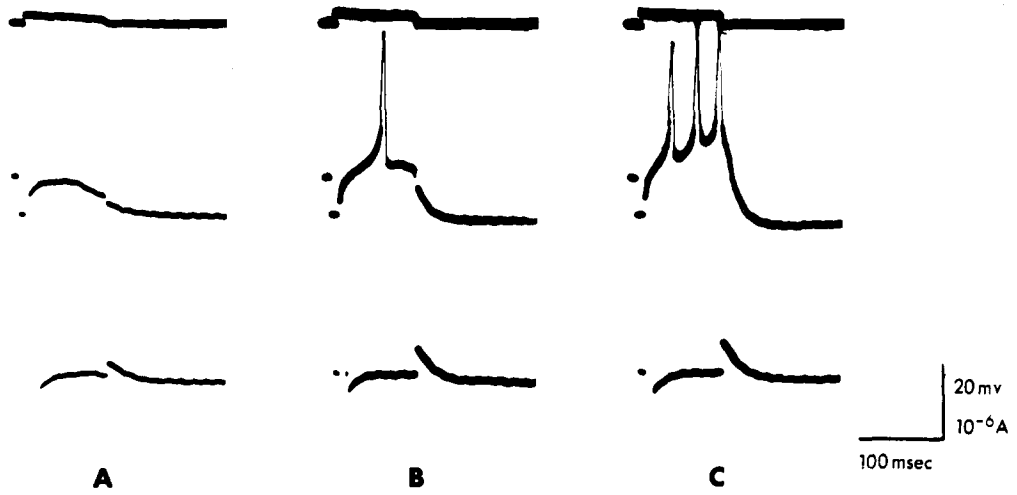

FIGURE 5 Conversion of fast division response to all-or-none spiking by treatment with TEA, 10^{-4} M. The preparation was in the recovery phase during washout with Na^+-free saline when this record was made. *A-C*, increasing intensity of depolarizing currents.

depolarizing quadrant, the line with slope = 2.5 kΩ fits satisfactorily. In other words, depolarization produces a drop in membrane resistance to about half the resting value. In lobster leg muscle, Werman and Grundfest (27) demonstrated that the depolarization-induced decrease in membrane resistance was due to increased K^+ conductance which could be blocked by treatment with tetraethylammonium ion (TEA). The same is true of the fast units of the remotor. Muscles in saline containing 10^{-4} g/ml TEA respond to depolarizing currents with prolonged overshooting action potentials. The effect is reversible and intermediate states of inactivation of K^+ conductance may be observed. Fig. 5 shows an instance where the muscle had been soaked in TEA saline, had developed prolonged spike responses, and was then returned to saline without TEA. In Fig. 5 A a subthreshold depolarizing current was passed through the membrane; the resulting depolarization indicates that the effective membrane resistance has been raised to about 100 kΩ. A larger current pulse in Fig. 5 B elicited an all-or-none spike, and a still larger current in Fig. 5 C produced a brief train of spikes. (The lowermost trace in each instance monitors the motor nerve to insure that firing of the nerve does not occur under these stimulus conditions.)

The responses shown in Fig. 5 occurred in saline containing the normal Ca^{++} concentration but lacking in sodium. The prolonged spikes produced in TEA saline were also insensitive to Na^+ concentration but were a function of the Ca^{++} concentration. Fig. 6, showing data taken from several experiments, is a plot of the peak amplitude of the TEA spikes as a function of the fraction of the normal Ca^{++} concentration present in the bathing saline. The slope indicated by the points appears to be less than the 29 mv per decade expected if Ca^{++} were the sole ion contributing to the peak depolarization.

Reuben (19) and Parnas and Atwood (16) have reported electrotonic current spread laterally among cells of crustacean muscles. In the latter instance the anatomical boundaries of cells were indistinct and the term subunit was adopted to denote what appeared to be an individual fiber. The same appears to be true in the remotor. The surface of the muscle viewed under low-oblique illumination presents the appearance of 800–1000 μ bundles of 100–150 μ fibers; the latter are separated by fine lines, the former by prominent longitudinal clefts. In Fig. 7 are records showing the spatial attenuation laterally and longitudinally of the potential changes produced by hyperpolarizing current pulses. The records in Fig. 7 a, b, and c were made with current and potential electrodes separated by approximately 50, 500, and 1000 μ, along the length of a single 150 μ wide subunit (the distances were estimated by holding a scale in the field near the electrode tips). The space constant in this direction is 1.4 mm in this case.

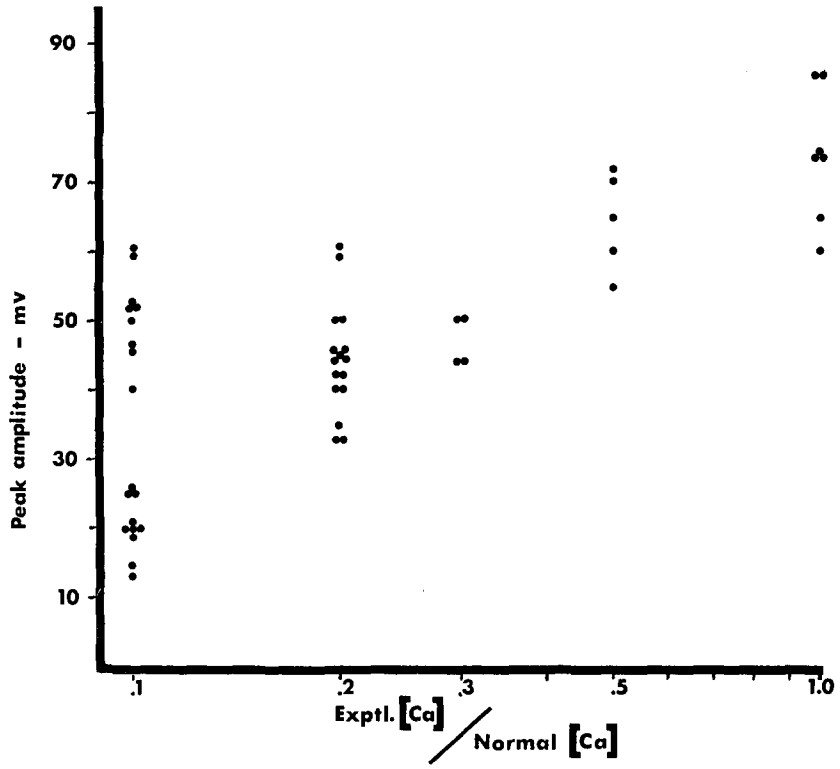

FIGURE 6 Dependence of TEA-induced spikes on Ca^{++} concentration. The peak amplitudes of prolonged TEA spikes are plotted as depolarization from resting potential.

The record in Fig. 7 d was made with the potential recording electrode in the same subunit as the current electrode and about 25 μ away from it (note the change in current calibration). In the record in Fig. 7 e the potential recording electrode was four subunits lateral to the current electrode, a distance of about 500 μ, but still in the same bundle of subunits; and in the record in Fig. 7 f the potential electrode was at the opposite edge of the bundle from the current electrode, a distance of almost 1000 μ. The decrease in the potential from d to f is only 40%. When the potential electrode was moved across the prominent boundary into the next bundle, the coupling disappeared as shown in the record in Fig. 7 g.

Rosenbluth (20) has found that delineation of the boundaries of individual fibers of the fast division is difficult, as in the crayfish deep extensor abdominalis medialis muscle (16), but says that syncytial units are several hundred micra across. I have therefore attempted to delimit the membrane-bounded units of the muscle by iontophoretic injection of two different dyes, Fast Green and Procion Yellow. The results with the two dyes were indistinguishable. With the use of currents of 0.5–1 μa for 2–5 hr it is possible to produce a dye mark that has spread 800–1000 μ from the electrode tip in the longitudinal direction; but the lateral spread does not exceed 150–200 μ in the surface plane, and dissection of the muscle after fixation in formalin shows penetration to about the same distance into the depth of the muscle.

Innervation of the Remotor Complex

The fast division of the remotor is supplied with two excitatory motor axons. Staining with methylene blue shows the two axons running together into the muscle at the cleft where the slow division fibers fan out from the apodeme. When the muscle mass is split open in the plane of the apodeme the nerves can be traced as they branch together repeatedly. Although the axons are nearly equal in size ($\simeq$ 50 μ diameter) at their entry into the

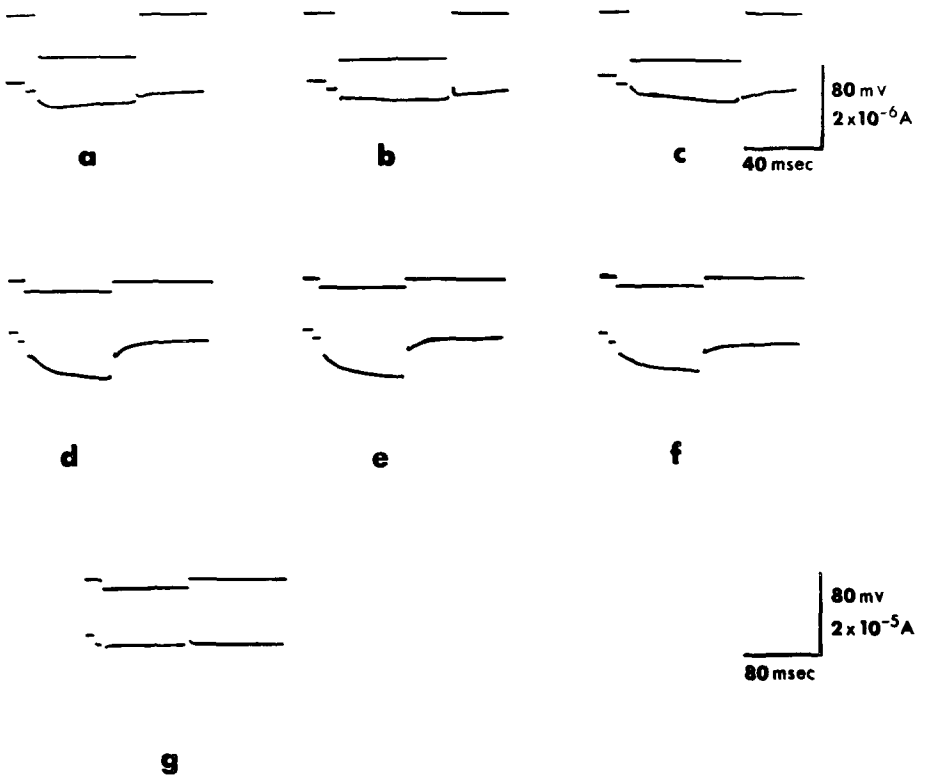

FIGURE 7 Longitudinal (a-c) and lateral (d-g) spread of injected current in fast division. The electrodes were separated by 50 μ in *a*, 500 μ in *b*, and 1000 μ in *c*. Records *d*, *e*, and *f* were taken with both electrodes in the same muscle unit, separated by 25, 500, and 1000 μ, respectively. In *g*, the current and potential electrodes were about 1000 μ apart but in neighboring units. The upper calibration refers to *a-c* only.

muscle, one of them becomes thinner than the other, once inside the muscle.

The first major branches run along the length of the muscle and at 300–350 μ intervals give off secondary branches that course transversely, rebranching repeatedly and disappearing from sight among the muscle units. No nerve branches were visible on the external surface of the fast division, all the endings being on inward-facing surfaces of the units.

The slow division also receives two fibers from the nerve. Both fibers are thinner than those to the fast division: one is 10–12 μ in diameter and the other is only 3–4 μ. The two fibers branch dichotomously with greater regularity than in the fast division and resemble more the situation in crayfish muscle (11). Fine endings and sprays occur all over the superficial surface of the fibers.

Cross sections of the entire nerve bundle before its entry into the muscle show two large fibers, one medium fiber, and three or four small fibers. The two largest fibers are the ones to the fast division; the medium fiber and one of the small fibers innervate the slow division; the destination of the other

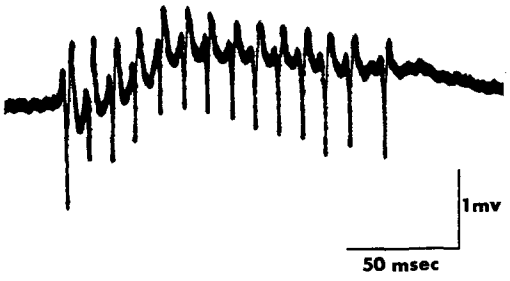

FIGURE 8 Electromyogram from bipolar electrodes chronically implanted in remotor of lobster. The signal coincided always with a palpable buzz when the animal was annoyed.

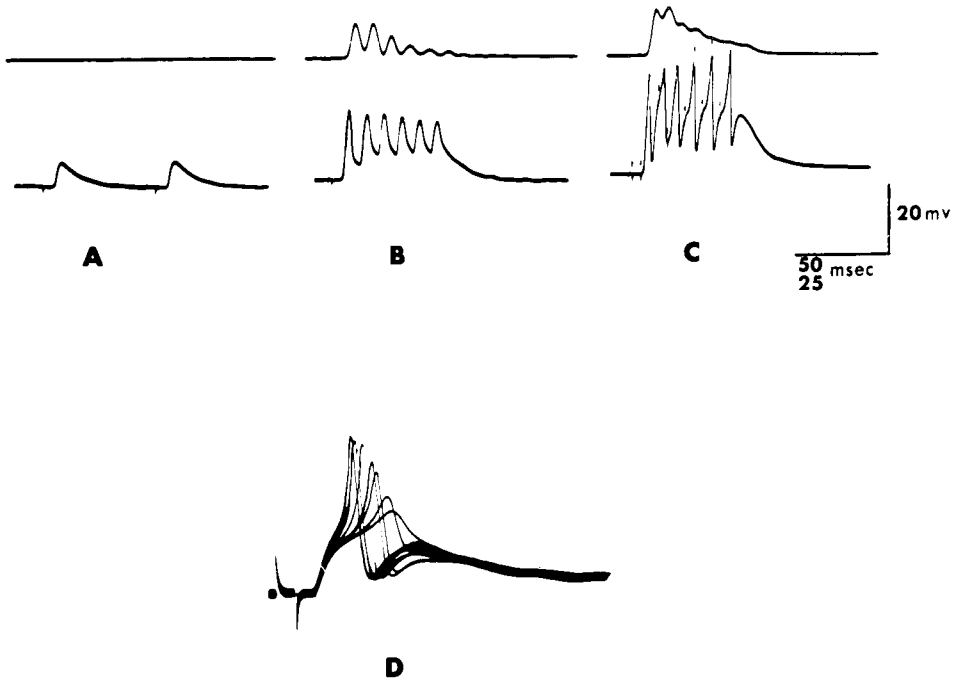

FIGURE 9 Responses of fast division units to stimulation of axon I. Upper trace in *A-C* registers whole muscle tension (increase upward); lower trace is transmembrane potential. In *D*, eight successive sweeps are superimposed. Further explanation in text. (25 msec calibration applies to *D* only.)

small fibers is unknown. They do not seem to be mechanosensory.

Responses of the Fast Division to Neural Input

Fig. 8 shows an electromyogram taken with chronically implanted electrodes which subsequent dissection showed to be in the remotor muscle. The lobster was prodded about the head and responded with a "buzz" that occurred simultaneously with the production of a burst of potentials from the remotor. Except for the final one, the intervals between potentials are all very nearly the same: 10 msec. Repeated elicitation of responses, even on different days, gave essentially the same result each time. Note that the first potential is the largest, and that the peak-to-peak amplitude then diminishes only to increase again toward the end of the burst. The remotor can obviously respond electrically to high-frequency neural inputs.

Electrical stimulation of the nerve bundle to the excised muscle confirmed the presence of two exciter motor axons, I and II, to the fast division, and one exciter motor axon, III, to the slow division. (These axons are numbered in order of increasing threshold.) The records of Fig. 9 A–C show the transmembrane potential (lower trace) of a fast division unit and the isometric tension (upper trace) produced by the whole muscle when axon I was stimulated at several different frequencies. In Fig. 9 A, at a stimulation rate of 20/sec the response consists of excitatory junctional potentials (ejp) of 7–9 mv lasting 15–20 msec, but no tension is apparent. Then the axon was stimulated with trains of pulses; the trains were delivered once every 2 sec and the pulse frequency within each train was 100/sec. Repeated presentation of stimuli in this manner caused a latent facilitation of the ejp's that built up from train to train (28). Fig. 9 B shows the response to one such train when the facilitation was well developed. The first ejp is increased in amplitude and repolarizes rapidly, a sign of electrically excitable activity of the membrane. The succeeding potentials are of lesser height and slightly longer time course, probably

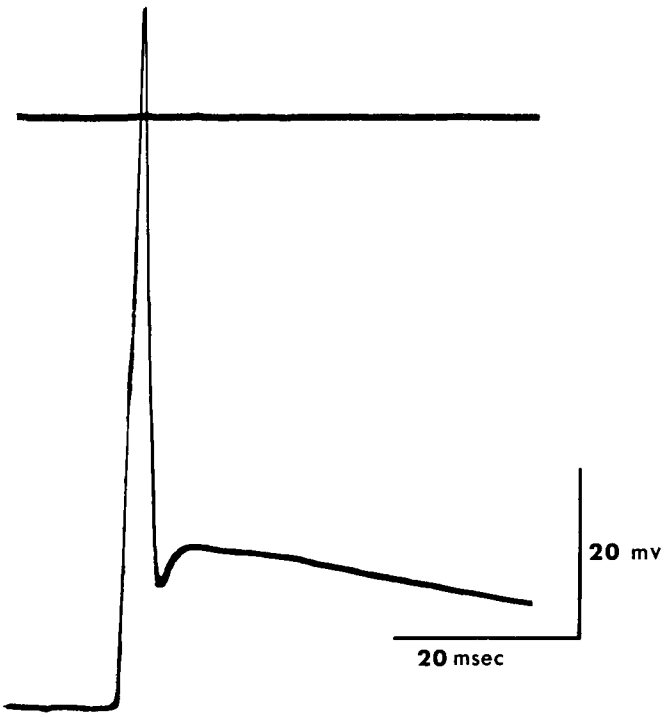

FIGURE 10 Response of a fast division unit to stimulation of axon II. The upper line denotes zero membrane potential. Usually the response is smaller and does not overshoot; only fresh, rested preparations give potentials as large as this one.

because of fatigue at the junctions overriding the facilitation. The tension produced by this response is a series of twitches of rapidly declining amplitude and showing little fusion. Fig. 9 C is the result of a similar stimulus, except that the frequency of stimuli within the train was 200/sec. Either the nerve or the muscle was unable to respond at this rate, and the recorded potentials recur at only about 140/sec. Here the facilitation is quite pronounced, the electrically excitable component is more strongly activated and the individual responses become more spike-like. The tension response is curious, indeed; fusion is evident at this high frequency of response, but the tension drops rapdily despite the progressive increase in the amplitude of the electrical response. Fig. 9 D, taken from another preparation, shows perhaps more clearly the course of facilitation and the corresponding increase in the graded electrical response of the muscle membrane. The record consists of seven traces superimposed one upon the other. Each trace commences with an artifact as axon I is stimulated. The response to the first stimulus produces the smallest and longest-lasting response. Succeeding stimuli occur every 10 msec; each stimulus produces a slightly larger, faster rising ejp which elicits a greater electrical response from the membrane to produce higher peak amplitudes and faster repolarizations during which the membrane conductance increases sufficiently to notch the declining phase of the ejp.

The electrical response of the fast division to stimulation of axon II in a fresh, rested preparation is shown in Fig. 10. (The line at the top indicates the zero potential level.) There is a very rapid depolarization inflected slightly about 50 mv below the resting potential, and an almost equally fast repolarization. The entire response has a peak amplitude of nearly 110 mv and a total duration, not including the tail of potential which may well be mostly contraction artifact, of 3.5–4 msec. As the preparation deteriorates, the response grows smaller and more prolonged. Fig. 11 shows the relation of tension production to electrical activity in response to axon II. The upper trace records membrane potential (at reduced gain) and the

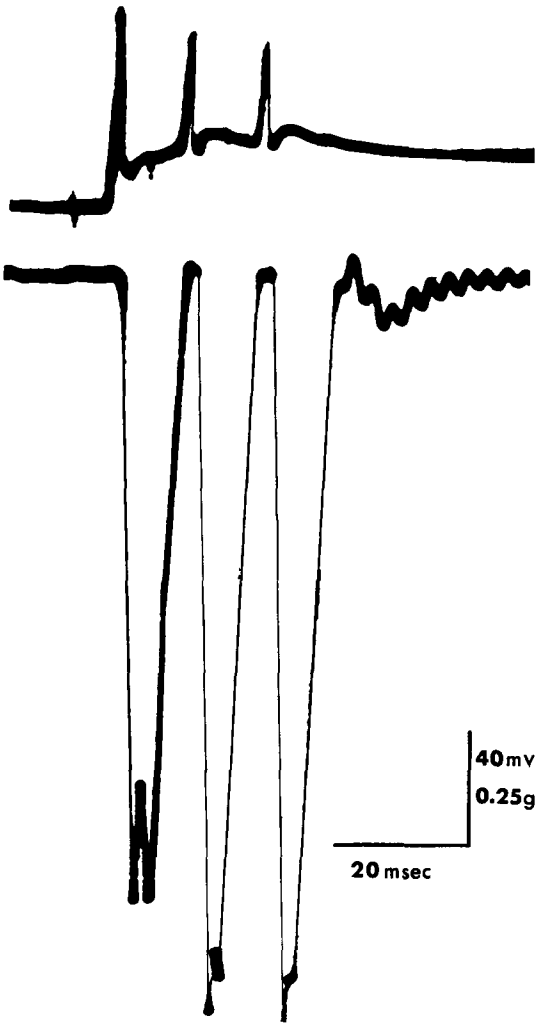

FIGURE 11 Electrical (upper trace) and mechanical response of fast division to three stimuli delivered to axon II. In this case the electrical responses did not reach zero potential. (Tension increases downward).

lower trace shows the resultant tension measured with an RCA 5734 transducer. Three stimuli were delivered to axon II at intervals of 11 msec. Longer trains of stimuli were avoided since they usually produced rapid fatigue of the responses. Each muscle potential is followed, within 2–3 msec of the onset of the depolarization, by a twitch of 1.5–1.7 g at optimal length, with rise time of 2.5–3 msec and relaxation time of 6.5–7 msec. Thus the entire twitch is complete in just under 10 msec. (The notch at the peak of the first twitch is seemingly due to resonance in the mechanical linkage and the RCA 5734 transducer since the interval from tension takeoff to notch is approximately the same as the period of free oscillation when the setup is tapped lightly [apparent also after the third twitch].) The decline in the height of the muscle potentials is very probably due to damage around the electrode tip which usually is expelled from the muscle during response to axon II.

It is clear from records such as this that the remotor can easily produce repeated contractions at frequencies up to 100/sec. The brevity of the twitches is dependent upon the repolarization of the muscle membrane. This is difficult to demonstrate directly because little tension is produced by direct, controlled depolarization uncomplicated by neural activation. Nonetheless, it is possible to see with the dissecting microscope a region of local contraction around the electrode tip during passage of large depolarizing pulses, and the lower trace of Fig. 12 shows a record of a just discernible

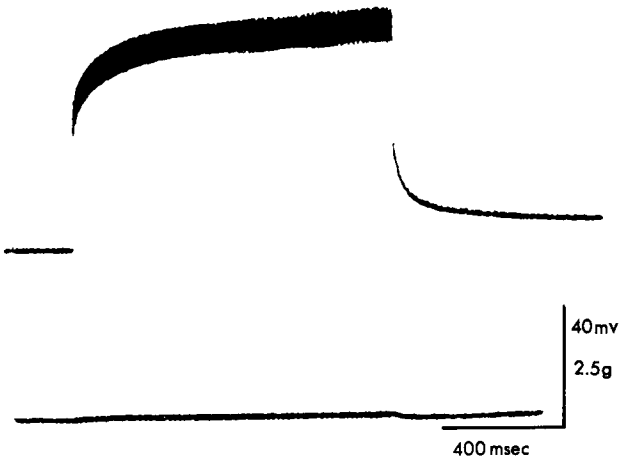

FIGURE 12 The lower trace displays the mechanical response of fast division to the current-induced depolarization shown on the upper trace. The tension of the whole muscle was measured but only a single unit was depolarized. The small active tension is maintained throughout the time of depolarization.

tension increase during the large depolarization shown on the upper trace. As long as the depolarization lasts the tension persists, relaxation occurring only upon repolarization. No signs of peripheral inhibition were noted in the fast division, but no specific effort was made to find such signs.

Responses of the Slow Division to Neural Input

Fibers of the slow division exhibit consistently larger input resistance than units of the fast division (Fig. 4, filled circles). They show no electrically excitable responses. Fig. 13 A shows the response of a slow division fiber to repetitive stimulation of axon III at 55/sec. It consists of a series of junction potentials which grow progressively larger by facilitation and summate slightly toward the end of the train. The tension begins to increase only after 50–60 msec and rises slowly until the stimuli end, after which it subsides very slowly indeed, half times for relaxation of the slow division ranging from 3 to 5 sec. Fig. 13 B shows the effects of higher stimulus frequency and latent facilitation from a previous activation: the ejp's start out larger and summate more to give a greater average depolarization. The tension rises faster to a greater amplitude than in Fig. 13 A. At optimal length, the slow division produces maximal isometric tension of 3.5–4 g. Activity produced by the second, thinner nerve fiber was not noted.

Synchronization in the Fast Division

One last observation requires mention here. In order to produce the sharply separated twitches shown in Fig. 11, the units of the fast division must be activated with very little temporal dispersion. This is only possible if all the nerve-muscle junctions are activated at nearly the same time by each impulse. Fig. 14 A superimposes simultaneous recordings from two microelectrodes inserted at opposite corners of the fast division as far from each other as possible. The electrically excitable responses were greatly depressed in this preparation and nearly pure ejp's are disclosed. The difference in time of onset of the ejp's at the two sites is less than 0.5 msec! On the other hand, as shown in Fig. 14 B, the temporal dispersion in the slow division can easily be 2–3 msec.

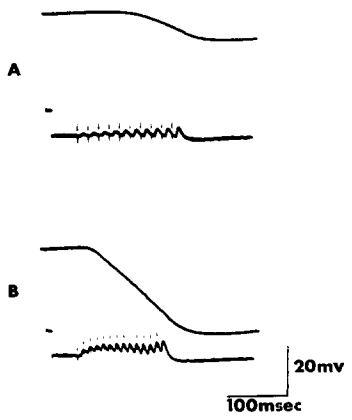

FIGURE 13 Responses of the slow division to stimulation of axon III at 55/sec in *A* and 90/sec in *B*. The upper trace in each depicts tension (increase downward) and the lower trace shows intracellular potential.

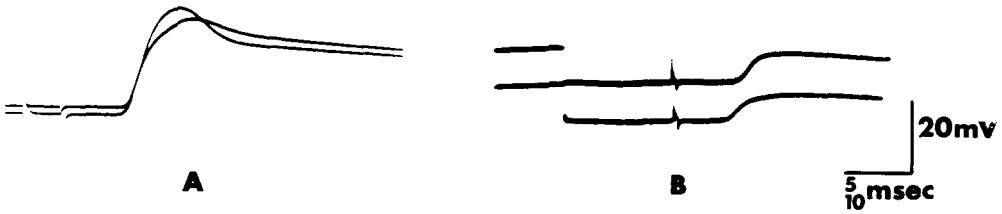

FIGURE 14 *A*: records from two recording electrodes in fast division. *B*: records from two recording electrodes in slow division. Full explanation in text. The 5 msec calibration refers to *A*, the 10 msec to *B*.

DISCUSSION

Mechanics

The experiments described in this report show that the remotor muscle of the lobster antenna is capable of contracting without mechanical summation at rates up to 100/sec, which is adequate to produce the underwater sounds Fish (7) recorded from lobsters. This muscle is the fastest synchronous muscle reported among the crustacea; indeed, it comes close to the performance of some of the fastest vertebrate muscles such as the sonic muscles of toadfish (23) and squirrelfish (8). This is impressive in view of the fact that the remotor achieves these speeds at 16–18°C, whereas the measurements on squirrelfish sonic muscle were done at 25–30°C, and on toadfish at 21°C. (Attempts to work with the remotor at such elevated temperatures were uniformly unsuccessful; above 20–21°C the preparations deteriorated far too rapidly for useful measurements.) Variation in temperature affects especially the rate of relaxation of muscle (18); the Q_{10} of the relaxation rate in frog gastrocnemius between 10°C and 20°C ranges up to 2.5; in the range 5–15°C, the Q_{10} of relaxation rate reaches values over 3.4 (15). It is possible, and reasonable, that the process responsible for this temperature dependence is the uptake of Ca^{++} from the myoplasm by the SR (21). In this light, the elaborate SR of the remotor muscle may be an adaptation that permits this muscle to operate at high rates at temperatures at which a muscle with the common amount of SR would be severely slowed.

It is not yet proved that the elaborate SR is indeed responsible for the rapid termination of tension in each twitch, although the connection is glaringly obvious. It is possible, however, to extrapolate from what is known about SR uptake of Ca^{++} to the situation in the remotor. This has been done by W. G. Van der Kloot and his calculations and conclusions appear as an appendix to this report. His results speak for themselves.

In order to be capable of high twitch rate, the lobster remotor has traded off strength, as is readily shown by comparison of the fast and slow divisions of the muscle. At 18°C and optimal length, the slow division can develop isometric tension of 2.8 kg/cm^2, which is well within the range common for most muscles. By comparison, the fast division under the same conditions develops isometric tension of only 25 g/cm^2. Allowance must be made for the fact that the fast division cannot be tetanized, but if a twitch-tetanus ratio of 3 is assumed for the fast division, a generous assumption, then the slow division can exert 35 times the tension of the fast division. Although other features of the organization of the muscle must be involved, a part of the disparity is due to the fact that myofibrils occupy only one-fourth of the fast division cross section. Another factor in the tension difference is probably the difference in thin-thick filament ratios in the two divisions. Auber (3) has already pointed out the decline in thin-thick filament ratio that accompanies increased speed in insect muscles, and the situation here seems in accord with that trend. Just how an increase in speed may be related to a decrease in thick-thin filament ratio is not clear at present, but it does seem likely that a drop in tension capability would be a consequence since the opportunity for cross-link formation (21) should diminish when fewer thin and thick filaments lie in close proximity to one another.

Besides functioning in the uptake of Ca^{++}, the SR may serve another function no less important even if incidental. This function may be to decrease the internal viscosity of the muscle. Although no confirmatory measurements exist, it is likely that most of internal viscosity is due to the closely packed myofilaments and that a lot of water-

filled spaces, as occur in the fast division, will offer much less opposition to rapid movement. Briefly put, in a muscle with low internal viscosity, the external tension should follow the active state tension with less time lag because the change in length of the sarcomeres, necessary for change in length of the series elastic component, can proceed faster. Confirmation of this possibility by direct determination would be most interesting, particularly since viscous drag should exert its effect primarily during relaxation when the restoring force moving the muscle is decreasing continuously with time. Since the remotor does not operate isometrically but must always move back and forth, a reduction in viscosity would be important.

Electrophysiology

The electrophysiology of the remotor is not unusual. The membrane responds to depolarization with graded depolarizing electrogenesis, apparently by increasing permeability to Ca^{++}, as has been shown to occur in barnacle muscle (10), and in crayfish (1) muscle. The response is graded rather than all-or-none, apparently because of a rapidly developing outward K^+ flux such as was shown to occur in lobster walking leg muscle by Werman and Grundfest (27). There, as with the remotor, TEA decreased the resting membrane conductance and caused production of all-or-none spikes apparently due to block of the K^+ activation mechanism. Similar results were also obtained with grasshopper muscle (5).

If there is anything striking about the electrophysiology of the remotor, it is the degree to which the remotor resembles the sonic muscle of the squirrelfish (8). Both muscles are multiterminally innervated, both are gradedly electrically responsive to excitatory ejp's, and both respond only rarely and poorly to intracellular depolarization via a microelectrode. The last was attributed by Gainer et al. (8) to damage caused by the microelectrode. In the experiments on the remotor, this was equally, if not more likely. As the current-voltage characteristic of Fig. 4 shows, the effective input resistance of the remotor is very low, of the order of 5kΩ, even when the resting potential is 70–80 mv. To produce appreciable voltage changes required large currents and current electrodes with large tips. Thus the very area of membrane to be stimulated by extrinsic current is that area most likely to be damaged, whereas the nerve junctions affect primarily undamaged membrane. Attempts were made at massive extracellular stimulation, but the action of the nerve could not be eliminated without eliminating the muscle membrane as well.

Gainer and Klancher (9) point out that in the squirrelfish and toadfish sonic muscles, multiterminal innervation substitutes the rapid conduction of nerve for the much slower conduction of muscle to secure simultaneous activation of the muscle, and obviously the same is true of the remotor. Yet in all three cases there exist mechanisms in the muscle for an active electrical response to junctional depolarization, a response which ordinarily serves to spread excitation and which is superfluous in that respect. It might be argued that electrical excitability is the common condition of vertebrate muscle and has simply persisted in the fish sonic muscles. However, electrical excitability is not the common condition in crustacean muscles. I suggest that, in the remotor, electrical excitability serves to guarantee rapid repolarization of the muscle between stimuli since, like so many other muscles, the remotor cannot relax within 6–10 msec otherwise. The remotor muscle does not have a fast, built-in relaxing mechanism such as is present in insect indirect flight muscle (see reference 17), yet intermittent relaxation is a sine qua non of sound production.

One last aspect of the operation of the fast division remains to be explained: Why is there a dual innervation? Generally, in crustacean muscle, dual or multiple innervation either serves to provide for the organization of more than one motor unit in the muscle or the different motor axons elicit mechanical activities that are quite different in magnitude and/or time course [reviewed by Atwood (2)]. From the available data, it appears that both motor axons of the remotor produce trains of rapid twitches, the only difference between the two being that axon II elicits full-size electrical responses and twitches beginning with the first impulse in a train, whereas axon I must fire several times before its junctions facilitate enough to provoke any graded responses at all. However, since transmission from axon II fatigues rapidly whereas that from I remains at the facilitated level for much longer, it seems at this point that the two axons are used in concert, i.e. axon II starts the train of twitches promptly, and axon I assures continuance of this train after II has begun to fatigue.

The EMG record in Fig. 8 supports this idea.

The first muscle potential is the largest; there follow a decline in peak-to-peak amplitude and finally, a return to intermediate size. It is difficult to account for this pattern other than by the operation of the two motor neurons as proposed above.

There yet remains the problem of elucidating the behavioral role of the lobster's sonic output. Surely so specialized a system must serve an important function in the life of the species.

The author wishes to express his appreciation to M. Leffert and R. D. Glantz who helped with the work on TEA and mechanics. Miss Lisbeth Billeter provided unfailingly valuable technical assistance. This work was supported in part by U.S.P.H.S. grant NB-04588 to the author and NONR-4785(00) to the Marine Biological Laboratory.

Received for publication 22 October 1968 and in revised form 8 April 1969.

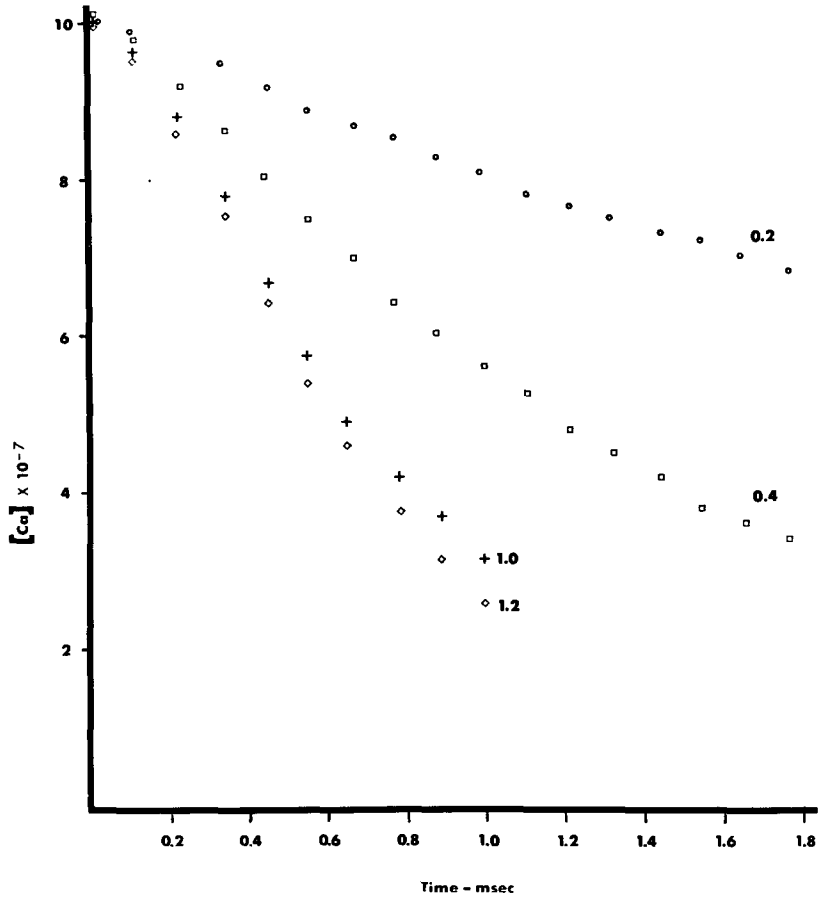

FIGURE 15 Calculated [Ca^{++}] at a point 0.1 μ from the center of a myofibril as a function of time. At $t = 0$, the sarcoplasmic reticulum begins to sequester Ca^{++}. Calculations for four different widths of SR collar are shown (○ = 0.2 μ, □ = 0.4 μ, + = 1.0 μ, ◇ = 1.2 μ). The assumptions underlying the calculations are given in the text.

APPENDIX

CALCULATED EFFECTS OF SR THICKNESS ON SARCOPLASMIC CALCIUM CONCENTRATIONS

WILLIAM VAN DER KLOOT. From the Department of Physiology and Biophysics, New York University School of Medicine, New York 10016

The question is whether an increase in the extent of the collar of SR would have any marked effect on the rate at which Ca^{++} could be pumped out of the actomyosin lattice. The calculations are based on the following assumptions: (*a*) The myofibril is a uniform cylinder 1.0 μ in diameter. (*b*) The myofibril is surrounded by a collar of SR; in the calculations the collar thickness is varied from 0.2 μ to 1.2 μ. (*c*) The outer edge of the collar abuts the boundaries of other identical cylinders, in which identical events are going on. (*d*) At time $t = 0$, there is a uniform $[Ca^{++}]$ throughout the system. At this moment the SR begins to take up Ca^{++}. The uptake rate depends on $[Ca^{++}]$, a rate constant, and the amount of SR present (24, 25). (*e*) Ca^{++} diffuses with a rate constant 6×10^{-6} cm^2/sec, about that in free solution.

In the calculations the [Ca] at intervals after t_0 was calculated at a series of points, 0.1 μ apart, along the radius of the cylinder, by a numerical solution of the diffusion equation with use of a difference method (13). The effect of changes in SR thickness on the rate of clearing of calcium from the sarcoplasm is shown in Fig. 15. The $[Ca^{++}]$ at a point 0.1 μ from the center of the myofibril is plotted as a function of time at four different widths of SR collar: 0.2, 0.4, 1.0, and 1.2 μ. An increase in the size of the SR does have a pronounced effect on the rate at which the $[Ca^{++}]$ within the myofibril falls. Increases in the extent of the collar above 1.0 μ have relatively little effect.

The large amount of sarcoplasmic reticulum in the remotor muscle therefore is probably significant in promoting rapid relaxation.

This work was aided by grant NB-04874 from the U.S. Public Health Service.

REFERENCES

1. ABBOTT, B. C., AND I. PARNAS. 1965. Electrical and mechanical responses in deep abdominal extensor muscles of crayfish and lobster. *J. Gen. Physiol.* **48**:919.
2. ATWOOD, H. L. 1967. Crustacean neuromuscular mechanisms. *Amer. Zool.* **7**:527.
3. AUBER, J. 1967. Distribution of the two kinds of myofilaments in insect muscle. *Amer. Zool.* **7**: 451.
4. BARBER, S. B., and W. H. MOWBRAY. 1956. Mechanism of sound production in the sculpin. *Science.* **124**:219.
5. CERF, J. A., H. GRUNDFEST, G. HOYLE, and F. V. MCCANN. 1959. The mechanism of dual responsiveness in muscle fibers of the grasshopper *Romelia microptera*. *J. Gen. Physiol.* **43**: 377.
6. FATT, P., and B. L. GINSBORG. 1958. The ionic requirements for the production of action potentials in crustacean muscle fibers. *J. Physiol. (London).* **142**:516.
7. FISH, J. F. 1966. Sound production in the American lobster, *Homarus americanus* H. Milne-Edwards (Decapoda Reptantia). *Crustaceana.* **11**:105.
8. GAINER, H., K. KUSANO, and R. F. MATHEWSON. 1965. Electrophysiological and mechanical properties of squirrelfish sound-producing muscle. *Comp. Biochem. Physiol.* **14**:661.
9. GAINER, H., and J. E. KLANCHER. 1965. Neuromuscular junctions in a fast contracting fish muscle. *Comp. Biochem. Physiol.* **15**:159.
10. HAGIWARA, S., S. CHICHIBU, and K. I. NAKA. 1964. The effects of various ions on resting and action potentials of barnacle muscle fibers. *J. Gen. Physiol.* **48**:163.
11. VAN HARREVELD, A., and C. A. G. WIERSMA. 1937. The triple innervation of crayfish muscle and its function in contraction and inhibition. *J. Exp. Biol.* **14**:448.
12. MENDELSON, M. 1966. Mechanical and electrical properties of a sound-producing muscle in the lobster *Homarus americanus*. *Amer. Zool.* **6**:539.
13. MCCRACKEN, D. D., and W. S. DORN. 1964. Numerical Methods and Fortran Programming. John Wiley & Sons, Inc., New York.
14. MOULTON, J. M. 1957. Sound production in the spiny lobster *Panulirus argus* (Latreille). *Biol. Bull. (Woods Hole).* **113**:286.
15. OSGOOD, P. F., and W. R. BREWSTER, JR. 1963.

Effect of temperature and continuous stimulation on gastrocnemius of the frog. *Amer. J. Physiol.* **205**:1299.

16. PARNAS, I., and H. L. ATWOOD. 1966. Phasic and tonic neuromuscular systems in the abdominal extensor muscles of the crayfish and rocklobster. *Comp. Biochem. Physiol.* **18**:701.
17. Pringle, J. W. S. 1965. Locomotion: flight. *In* The Physiology of Insecta. Academic Press Inc., New York.
18. RAMSAY, R. W. 1960. Some aspects of the biophysics of muscle. *In* The Structure and Function of Muscle. G. H. Bourne, editor. Academic Press Inc., New York.
19. REUBEN, J. P. 1960. Electrotonic connections between lobster muscle fibers. *Biol. Bull. (Woods Hole)*. **119**:334.
20. ROSENBLUTH, J. 1969. Sarcoplasmic reticulum of an unusually fast-acting crustacean muscle *J. Cell Biol.* **42**:534.
21. SANDOW, A. 1965. Excitation-contraction coupling in skeletal muscle. *Pharmacol. Rev.* **17**: 265.
22. SCHMIDT, W. 1915. Die musculatur von *Astacus fluviatilis*. *Z. Wiss. Zool.* **113**:165.
23. SKOGLUND, C. R. 1961. Functional analysis of swim-bladder muscles engaged in sound production of the toadfish. *J. Biophys. Biochem. Cytol.* **10**:187.
24. VAN DER KLOOT, W. G. 1965. The uptake of Ca^{++} and Sr^{++} by fractions of lobster muscle. *Comp. Biochem. Physiol.* **15**:547.
25. VAN DER KLOOT, W. G. 1966. The exchange of radioactive cations by somatic and cardiac muscles in the crayfish. *Comp. Biochem. Physiol.* **17**:1019.
26. WERMAN, R., F. V. MCCANN, and H. GRUNDFEST. 1961. Graded and all-or-none electrogenesis in arthropod muscle. I. The effects of alkali-earth cations on the neuromuscular system. *J. Gen. Physiol.* **44**:979.
27. WERMAN, R., and H. GRUNDFEST. 1961. Graded and all-or-none electrogenesis in arthropod muscle. II. The effects of alkali-earth and onium ions on lobster muscle fibers. *J. Gen. Physiol.* **44**:997.
28. WIERSMA, C. A. G. 1961. The neuromuscular system. *In* Physiology of Crustacea. T. H. Waterman, editor. Academic Press Inc., New York.