

## THE CATCH PROPERTY OF ORDINARY MUSCLE\*

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Three background issues motivate the present study. First, it has long been known that a special category of muscle, found in certain molluscs, can maintain tension for long periods after brief excitation with very little energy expenditure.<sup>1-3</sup> Relaxation in these catch muscles may be separately initiated by inhibitory or relaxing neural input, by 5-hydroxytryptamine, by temperatures over 30°C, and perhaps by low Ca<sup>++</sup> concentration.<sup>4, 5</sup> Molluscan muscles contain large amounts of a special protein, paramyosin (tropomyosin A), which might be crucial in the maintenance of prolonged contractions,<sup>6, 7</sup> or it might only contribute to the unusually great tensile strength of the thick filaments.<sup>3</sup> During long-maintained contractions in intact animals there appears to be a constant, low level of neurally evoked electrical activity.<sup>8-10</sup> In spite of the large number of diverse experiments that have been performed on these special muscles, it is still said that "the basis of catch tension is not yet understood."<sup>4</sup>

Second, there are the phenomena we may call the Blaschko effects, observed in certain crustacean muscles.<sup>11</sup> Here, a single impulse in an excitatory motor axon to the crayfish claw closer muscle produces only a small contraction, while a low-frequency train of impulses gives rise to a slowly developed but much larger tension plateau. But a single extra impulse interpolated within this low-frequency train *produces a marked extra contraction that is maintained beyond the normal twitch duration*. If a brief high-frequency train of impulses is added to the low-frequency background, then the muscle may contract to a new tension plateau *from which it does not fully relax for long periods after the return to low-frequency stimulation*.

Finally, Partridge<sup>12</sup> has recently shown that a mammalian skeletal muscle preparation shows a slowly decaying hysteresis when the nerve is stimulated by sinusoidally frequency-modulated impulse trains. His technique did not, however, allow the localization of the site of the hysteretic process.

We feel that the evidence presented below suggests that these three categories of muscle response may be mechanistically related. We have chosen arthropod nerve-muscle preparations for the examination of Partridge's hysteresis effect because they make it relatively easy to work with single motor units and single muscle fibers. These preparations have not only exhibited the hysteresis phenomenon but have additionally allowed us to verify the old Blaschko effects and to relate them to modern studies. And our work with one type of insect muscle has also convinced us that the phenomena we have observed are not specific to crayfish muscle.

*Materials and Methods.*—The claw opener muscle of the crayfish (*Procambarus clarkii*) has one excitatory axon.<sup>13</sup> It can be selectively stimulated by electrodes placed under the thin bundle of the nerve coursing through the meropodite. Stimulation of that axon gives rise to small junctional potentials which may summate up to a 30-mv depolarization in all the muscle fibers. Summation is necessary before measurable tension changes

can be observed. Excitatory junctional potentials at the distal and proximal ends of the muscle facilitate strongly at low frequency, whereas those in the central region of the muscle facilitate markedly only at high-stimulus frequency. Under steady-state stimulation membrane, depolarization (up to 30 mv) appears to be almost linearly related to developed tension. Finally, the electrical space constant of the individual muscle fibers is greater than the fiber length; hence, electrical signals applied to one end of a fiber are not much attenuated during passive spread throughout the fiber length. This feature is important for our intramuscular stimulation experiment. (This summary of electrical properties is from Bittner.<sup>14</sup>) The opener muscle also receives one inhibitory axon via the thick bundle of the same nerve. Stimulating it produces both pre- and postsynaptic inhibition of excitatory axon effects, and hence decreased tension.<sup>15, 16</sup>

The closer muscle of the crayfish claw is innervated by two excitatory axons and one inhibitor.<sup>13</sup> One exciter gives rise to small twitches on single stimulation, the other produces large twitches. Repetitive stimulation produces tetanus and much increased tension.

The tibial extensor muscle of the jumping leg of the locust *Schistocerca gregaria* is innervated by two excitatory axons and one inhibitor,<sup>17, 18</sup> and these can be selectively stimulated by pin electrodes inserted appropriately into the ventral thoracic region. The fast exciter axon, which innervates almost all the muscle fibers, causes strong twitches with single impulses and fatigues rapidly on repetition. The slow exciter axon innervates only about 20% of the muscle fibers and repetitive stimulation is necessary to cause significant tension development.

We measured the mechanical activity of the muscles in several ways. For whole muscle preparations isometric tension was recorded by letting the dactyl of the crayfish claw or the tibia of the insect leg push directly on a stiff rod bearing a strain gauge (Bionix, Berkeley, Calif.). Isotonic recordings from the claw were made using another Bionix device with a very light lever. Nearly isotonic recordings from the insect leg were obtained by allowing the tibia to push against a long quartz fiber that was attached to the pin of an RCA 5734 transducer tube. Isometric recordings from single muscle fibers of the crayfish claw were made by gripping the apodeme of the muscle in forceps that were rigidly fixed to the pin of the RCA 5734 tube. The hysteretic phenomena we describe occurred at all magnitudes of muscle contraction, from those barely measurable to those of maximum amplitude.

Intracellular stimulation was performed and recorded via 3 M KCl-filled glass capillary microelectrodes. Most of the electronic apparatus employed in these studies was standard. Sinusoidally frequency-modulated impulse trains were obtained from a voltage-controlled oscillator (Wavetek, model 114) driven by another function generator (Exact Electronics, type 250). The Wavetek output triggered the Tektronix 160 series generators, which delivered variable voltage square-wave pulses of about 1 msec duration. Primary recordings were displayed on a Clevite-Brush lightbeam oscillograph and a Tektronix type 564 storage oscilloscope. The latter was often operated with the horizontal deflection driven by the same signal that modulated the stimulus train (see Fig. 2). The storage oscilloscope was photographed with Polaroid film. Since our recording techniques were of inherently rather low contrast, the records for the figures have been traced from the originals.

*Results.*—Figure 1 illustrates one of the Blaschko effects for the crayfish claw opener and closer, and insect tibial extensor muscles. A high-frequency train of stimuli in the middle of one of a long lower frequency increases the contraction dramatically, and the relaxation following the high-frequency stimulation is incomplete over the short term. Yet, when the stimulation is terminated altogether, the muscle quickly relaxes to resting length. The effect is less pronounced in the insect preparation than in the crayfish. If the stimulus is interrupted during the contraction plateau following high-frequency stimulation,

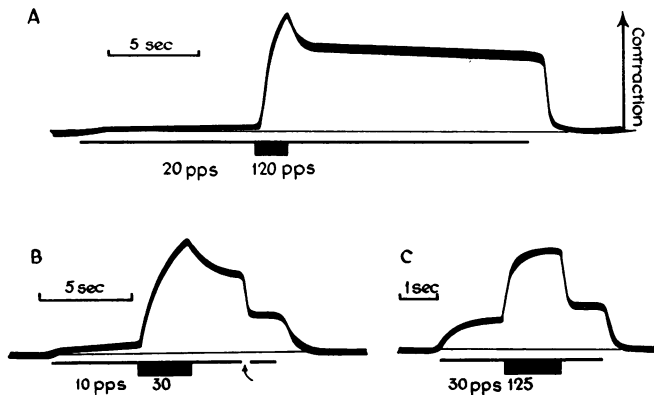


FIG. 1.—(A) Isotonic contraction of crayfish claw opener muscle. Stimulation at 20 pulses per sec (pps) produces only a slight movement. A brief increase to 120 pps results in a long-lasting reset to a much increased degree of contraction.

(B) A similar record for the claw closer muscle stimulated by its slow axon. During the “after” plateau, brief interruption of the low-frequency stimulation (arrow) results in rapid relaxation, but renewed low-frequency stimulation again catches the contraction.

(C) Similar, but not so conspicuous a behavior, is demonstrated by stimulating the slow exciter axon of the locust tibial extensor muscle.

rapid relaxation begins, but relaxation can be interrupted at any state by renewing the low-frequency stimulation (Fig. 1B). A given level of contraction can be held by a lesser degree of stimulation than that required to produce it in the first place, as though there were a ratchet mechanism in which little “nervous energy” is required to hold the catch.

The contraction plateaus illustrated in Figure 1 are seen to persist for a very long time relative to the unstimulated relaxation times. But do they represent a truly decayless hysteresis? Probably not, but this is not easily tested with these preparations. We have examined records from very long trains of stimuli where the “before” plateau of position or tension is in fact gradually rising, and the “after” plateau is gradually declining. They may be approaching the same level. Unfortunately, really steady-state conditions cannot be examined because fatigue interferes with obtaining the prolonged and stable records that are required. Other methods of stimulation and record display allow a better estimate of the duration of the hysteresis.

Figure 2A shows that the mechanical response which results from a sinusoidally varying frequency of input impulses is not a sinusoid: there is both a phase lag and a distortion even at quite low modulation frequencies. The distortion is more apparent when contraction magnitude and stimulus frequency are plotted as vertical and horizontal values, respectively, on a two-dimensional plot that lacks an ordinary time base (Fig. 2B). The latter type of record varies in shape depending upon numerous parameter changes: mechanical conditions (for example, whether contraction is isotonic or isometric), mean stimulus frequency, depth of pulse frequency modulation, and frequency of modulation. Figure 3, for example, shows the effect of differing modulation frequencies on the insect leg

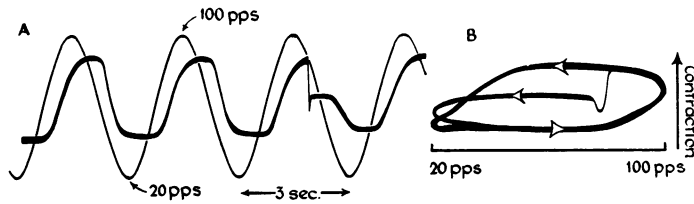


FIG. 2.—(A) Isotonic contraction of insect leg muscle excited by a sinusoidally modulated impulse train in the slow exciter axon. The muscle length record is superimposed upon the modulating sine wave. During the third cycle the muscle was passively lengthened and released quickly.

(B) The last two cycles of record (A) plotted, using the sine wave to drive the horizontal axis. The distortion is much clearer in this mode of data presentation.

preparation. The hysteresis loop is maximally open at about one cycle per ten seconds. At higher modulation frequencies the loop flattens because the muscle has not had time to relax during the lower-frequency portion of the stimulus cycle. At modulation frequencies of less than one cycle per minute the loop also flattens, but with a slope that may approach the steady-state frequency-tension relationship. A stimulus modulation cycle of four minutes was the most prolonged that was observed to produce an open loop record which repeated exactly for at least two consecutive cycles. However, we have obtained indications of hysteresis lasting at least ten minutes, but at these low modulation frequencies nerve threshold changes and other noise-producing factors make reliable sampling difficult. It does appear to be true, however, that the hysteresis is not permanent, and that in recordings such as those in Figure 1, "before" and "after" plateaus would approach each other after several minutes, at most.

The hysteresis was evident under both constant-length and constant-tension circumstances, but it was more pronounced in the latter case. The catch in muscle length had a slower decay than the tension catch.

It was impossible to demonstrate the usual hysteresis loops when stimulating the fast axon of the insect leg muscle preparation at low modulation frequencies. This was clearly because the fast neuromuscular transmission process fatigued

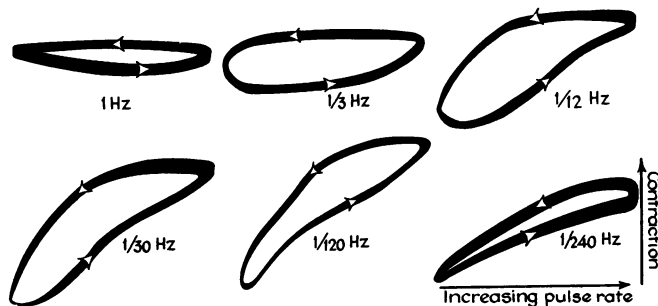


FIG. 3.—Hysteresis loops for isotonic contraction of the insect leg muscle at a series of modulation frequencies (indicated on the figure). The impulse train was varied from 30 to 90 pps.

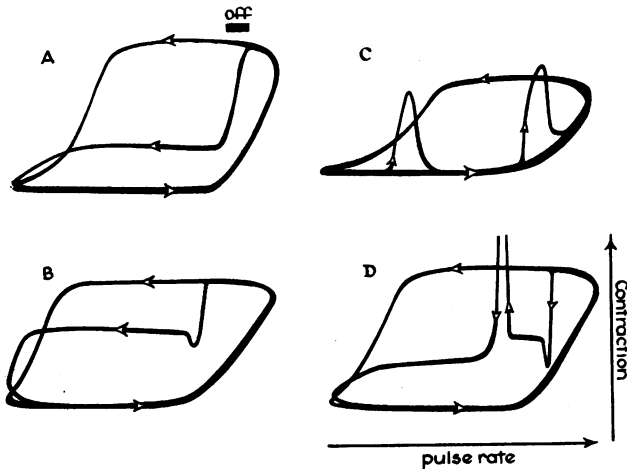


FIG. 4.—Hysteresis loops for isotonic contractions of the insect muscle stimulated by a train modulated from 10 to 100 pps at 0.1 Hz.

(A) A brief interruption of the stimulus train results in rapid relaxation until stimulation resumes.

(B) A similar curve results when the muscle is lengthened by an external force during continuous stimulation.

(C) An externally imposed shortening of the muscle does not cause resetting of its length.

(D) A length reset due to passive elongation cannot be followed by a reset to shorter length, even though the muscle could hold a shorter length under the identical stimulus history.

too rapidly with the high-stimulus frequencies and long periods of stimulation required in these experiments.

Figure 4 illustrates some effects of interfering with the preparation during the stimulus cycle. Turning the stimulus off briefly during the decreasing frequency half-cycle allows rapid relaxation until the stimulus begins again (Fig. 4A). The contraction then holds at a low level and fails to climb again toward the level that it would have held if the interruption had not occurred (compare with Fig. 2). Figure 4B shows that a similar effect is obtained when the muscle length is reset by external mechanical means (also shown in Fig. 2). Here, the length resetting was done by pushing rapidly on the claw or leg and releasing it quickly. (Note that there is a small rebound, presumably because internal viscosity has not allowed the muscle to relax fully to the new length.) Generally the record resembles that due to stimulus interruption (including a quite regular but unexplained overshoot of the normal loop at the low-frequency end of the cycle). Figure 4C illustrates the converse experiment which shows that pushing the leg in the direction of muscle shortening does not cause a length reset. Figure 4D shows that once the contracted muscle has been reset to a longer length it cannot hold a shorter length, even after being mechanically pushed in that direction; only if a higher-frequency stimulation is used can it again achieve a highly contracted state. There appears, therefore, to be a unidirectional catch or ratchet which requires only a low level of nervous excitation.

The results presented thus far demonstrate that a hysteresis similar to that

described by Partridge does occur in arthropod muscles excited via single motor axons. Hence, motor neuron recruitment could not explain the results. Neither muscle fatigue nor nerve or junctional fatigue (which might produce stimulus-frequency division) would produce such a hysteresis; in fact, these conditions might give rise to loops of opposite sense. However, the particular nerve-muscle preparations we have used do exhibit facilitation at the neuromuscular junctions, and facilitation could give rise to membrane changes lasting long enough at least to contribute to the effect.<sup>14</sup> Membrane potential records from various muscle fibers show different patterns of change during sinusoidally varying input frequency (Fig. 5). Some fibers facilitate so slowly that there is no hysteresis of the membrane potential after one or two modulation cycles, even at 1/10 Hz. Others show moderate hysteresis or complex loops due to varying degrees of facilitation at different frequencies. These results seem consistent with Bittner's finding that there is variety in the responses of different muscle fibers innervated by the single axon due to varied properties of the different pre-synaptic terminals (see *Materials and Methods*).<sup>14</sup> However, none of the membrane potential records shows a degree of hysteresis that could explain the tension records by a linear transduction model.

A more conclusive demonstration that the hysteresis is not simply a property of the neuromuscular transmission and electrical membrane processes is provided by the following experiments. Figure 6 shows the result of stimulating intracellularly and recording tension from the same single fiber while monitoring the transmembrane potential with a second electrode. An imposed current which resulted in a sinusoidal membrane voltage produced tension loops like those resulting from nerve stimulation. The mechanical reset phenomenon was demonstrated here too. That the tension loop was not due simply to slow relaxation was shown by suddenly returning the membrane potential to resting level; the tension then returned rapidly, too.

We have also reproduced the Blaschko effects with the claw closer muscle. Even with the slower of the two exciter axons, a single impulse interpolated into

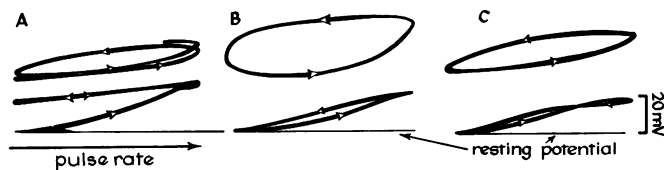


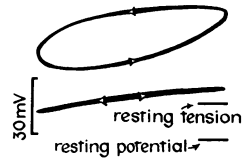
FIG. 5.—Examples of membrane potentials of three individual muscle fibers and isometric tension in the whole crayfish claw opener muscle during a sinusoidally modulated stimulation of the exciter axon. A low pass filter eliminated individual junctional potentials from the membrane potential records. Stimulus modulation was from 10 to 100 pps at 0.1 Hz.

(A) The membrane potential summates and facilitates on the first modulation cycle in this fiber.

(B) An example of the many cases in which neuromuscular facilitation gives rise to some hysteresis in the membrane potential record.

(C) Figure-eight loops (and even reverse loops) often occur in the membrane potential records of individual muscle fibers, even while the whole muscle exhibits the usual behavior.

FIG. 6.—Isometric tension in a single muscle fiber of the crayfish claw opener muscle shows hysteresis, even when the membrane potential is displaced by a current fed directly into the cell. Stimulation at 0.33 Hz shows that the membrane response itself is not hysteretic.



a long, low-frequency train gives rise to a large tension increase, but this increase usually lasts only about one second, not very much longer than an ordinary twitch in this muscle. The effect may, however, considerably outlast the correlated change in membrane potential. The Blaschko length-reset phenomenon, as seen in the case of interpolated high-frequency trains of impulses, was also found not to be correlated with an equal reset in membrane potential.

We have also tested the effects of neuromuscular inhibition on the contraction hysteresis in the claw opener preparation. A brief burst of inhibitory axon stimulation during the decreasing half of the exciter axon stimulus-frequency-modulation cycle has essentially the same effect as interrupting neural excitation or mechanically resetting the muscle length. A maintained constant-frequency inhibition depresses the whole loop, but does not fundamentally change its shape. If the exciter axon is stimulated at constant frequency while the inhibitor axon is stimulated with a sinusoidally modulated train, then a tension loop is also observed, but it is clockwise as opposed to the counterclockwise loops we otherwise obtain. Less complete evidence suggests that the inhibitor axon of the insect leg muscle has similar effects. All these results imply that inhibition does not have any special role relative to the muscle catch mechanism, but merely acts by subtracting excitation. Since the inhibitory axon produces both pre- and postsynaptic inhibition, we know that even the latter type has no special *catch-releasing* or *plasticizing* effect in these muscles.

*Discussion.*—It has been reported that membrane potential and tension are linearly related in the crayfish claw opener muscle when the measurements are made after four seconds of steady stimulation.<sup>14</sup> The present experiments suggest, however, that such measurements are inadequate to predict the muscle's behavior. The voltage-tension relationship is not simply linear, except perhaps in the nearly unattainable steady state and under other very special conditions. We see now that it is necessary to consider past stimulation and past load, and the direction of variation of these, in order to predict muscle action.

How general might the phenomenon be that we have described? In the crayfish, hysteresis was observed in the claw opener and closer muscles, in the superficial flexor muscles of the abdomen, and in the anal dilator muscle. We see it also in the insect leg muscle. Perhaps similar hysteresis has been reported in a barnacle muscle.<sup>19, 20</sup> We have already pointed out that hysteresis occurs in mammalian skeletal muscle,<sup>12</sup> and that many lamellibranch molluscs exhibit the relatively very long-term catch mechanism.<sup>2</sup> One can predict the presence of hysteresis in the behavior of frog and dogfish muscle from observations by Penuyck.<sup>21</sup> We have not been able to demonstrate a catch property in the most twitch-type arthropod muscle, but this may be due only to the fact that in the

muscle we examined the neuromuscular junction fatigues too rapidly. It seems likely, however, that "catchiness" is a general property of muscle, and that twitch muscles and clam catch muscles are extremes of a spectrum. However, we have not yet tested whether there is a catch mechanism in the energetic sense as opposed to neural control sense in these muscles, as there is in some molluscan ones.

Where is the catch mechanism? In lamellibranch molluscs it may reside in the protein contractile mechanism itself.<sup>3</sup> We can show in the crayfish muscle that it does not reside in the neuromuscular junction or sarcolemmal electrical processes, so perhaps it is the contractile machinery that is directly responsible here as well. Alternatively, it might be in the  $\text{Ca}^{++}$  binding mechanisms.

The behavioral significance of the above work is not altogether clear to us. It is true that it now appears even more difficult to explain animal movement in terms of detailed motor neuron output patterns, since the history of excitation, position, and load of each muscle must be considered in addition to properties already known to be important. Perhaps a catch mechanism in postural muscle could result in some energy savings. Whatever the teleology of the catch mechanism, it implies another need for proprioceptive reflex feedback for accurate muscle length control during animal movements (see also refs. 21 and 22). Since the phenomenon of catchiness depends upon present load and the mechanical history of the muscle, as well as the present and past motor output, purely central nervous motor scores cannot uniquely command even rather simple behavioral sequences.

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