The Basis for Prolonged Contractions in Molluscan Muscles

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ABSTRACT Two basically different hypotheses have been advanced to explain the behavior of molluscan muscles in cases in which relaxation of the muscle is extraordinarily prolonged. In one hypothesis, tetanic activation due to prolonged activity in an intrinsic ganglion network is postulated; in the other, changes in the mechanical properties of the muscle capable of maintaining tension generated by the contractile system are proposed. Experiments reported here were designed to test these hypotheses. Recordings were made of electrical activity in a number of circumstances in which the muscle relaxes slowly, and this activity was absent in some cases and in others was not found to correlate well with rate of relaxation. Quick release of the muscle during and after a stimulus which induced slow relaxation showed disappearance of the active state long before decay of tension. Contractile tension decreases with length below rest length whereas passive tension due to stretch following D. C. stimuli remains approximately independent of length. The latter has the same mechanical basis as prolonged relaxation following D. C. stimuli. Thus initial contractile tension and the tension remainder during prolonged relaxation appear to originate through different mechanisms. These results lead us to favor the second hypothesis above. A means by which this could be achieved in vivo is discussed.

INTRODUCTION

The contractile responses of many types of muscles, notably among the lamellibranches, differ considerably from responses shown by vertebrate skeletal muscles, in that the duration of the relaxation phase of contraction depends on the kind of stimulus applied. The anterior byssus retractor muscle (ARBM) of the sea mussel, Mytilus edulis, responds to direct current stimuli differently than it does to alternating or repetitive stimuli. The latter stimuli produce contractions which are followed by relatively fast relaxation, complete within a matter of seconds; whereas D. C. stimuli produce contractions of approxi-
mately the same magnitude, followed by prolonged relaxation, requiring minutes to hours (1, 2).

Several hypotheses have been proposed to explain these vast differences in the duration of relaxation. These are basically of two types. The first type postulates a change in the load-bearing elements within the muscle which would allow the muscle to remain contracted for long periods of time but which differs from the contractile process itself. Continued reactivation of the latter system would not be required for the maintenance of tension. The second type postulates continued reactivation during the relaxation phase of the contractile system by way of the neuromuscular system. According to the latter, relaxation is delayed for essentially the same reason that tension decays slowly in vertebrate skeletal muscles during reflex after-discharges; namely, that the active state of the contractile system is maintained by continued re-excitation derived from the nervous system.

A theory of the first type was proposed by Winton (1, 3) who, in attempting to explain differences in the plasticity of the ARBM following D. c. and A. c. stimuli, proposed that the former stimulus increased the viscosity of contractile elements, and that slow return to rest was due in this case to mechanical retardation of movements between these elements. Others have proposed that stimuli which bring about slow relaxation activate a "catch mechanism" in parallel with the contractile elements, similar to the mechanism proposed by Von Uexkull (4) to explain prolonged shell closure in bivalve molluscs. A physical model of such a system has been described by Bayliss (5). Other models have been based on analogies to changes in plasticity of actomyosin threads which occur when adenosinetriphosphate (ATP) is removed from the surrounding medium following contraction. Tension developed during contraction in ATP is maintained under such conditions by the change in stiffness of the fiber resulting from removal of ATP (see Weber (6)). Pryor has proposed a model of this type (7, 8). More recently, the protein paramyosin, extractable in large quantities from molluscan muscles, has been implicated in the catch mechanism (9).

The tetanic hypothesis has been proposed by Hoyle and Lowy (10), who have recently demonstrated the presence of continued electrical activity within the muscle during the period in which the muscle slowly relaxes following D. c. stimuli. They propose that relaxation is prolonged by continued reactivation of the contractile system by way of a neuromuscular activating system. Thus, according to this hypothesis, the difference between the behavior of the muscle following D. c. and A. c. stimuli resides in a difference in the amount of activity remaining in an intrinsic ganglionic plexus following cessation of the stimulus; the first type of stimulus thus induces an after-discharge in the neuromuscular system. This scheme implies that the unit response of the contractile system remains the same in both cases, whereas in
the first type of hypothesis, a qualitative difference in the basic response of the contractile system is postulated for contraction and for slow relaxation.

The importance of deciding between these two hypotheses and thus of deciding whether or not a tension-maintaining system other than tetanus is present in molluscan muscles has led us to examine certain further consequences of the "tetanus" theory. (a) If prolonged activity of the following neuromuscular system is responsible for slow relaxation, then one should be able to detect electrical activity within the muscle under all conditions in which prolonged relaxation follows contraction and which can be shown not to be due to other causes, such as depolarization contracture. (b) This electrical activity should bear a direct relation in intensity and duration to the tension developed or to the shortening of the muscle during the entire period of slow relaxation. (c) If prolonged relaxation is due to continued reactivation, then something analogous to Hill's "active state" (11) should be observable during this period, and the intensity of the active state should be related to the observed contractile activity; i.e., the active state should decay as slowly as or parallel to the decay of tension in an isometric contraction. (d) It will be shown in the following that tension disappears if the muscle is shortened slightly during the stage of prolonged relaxation, but that tension reappears if the muscle is restretched. This tension is far in excess of tension produced by comparable stretch at rest. If such reappearance of tension were due to a tetanic mechanism, one would have to postulate the presence of a system which was sensitive to the length of the muscle or to stretch, and in which activity ceased when the muscle was allowed to shorten, reappearing again as soon as the muscle was stretched. Reappearance of tension in excess of resting tension upon stretch of the muscle would be more easily explained by postulating a stimulus-induced change in the stiffness of the muscle, so that, during prolonged relaxation, the muscle would behave like a viscous-elastic body in which the elastic constants, and perhaps the viscosity, had been increased. This, along with other evidence, leads us to favor the first type of hypothesis given above.

Methods

A. MULTICHANNEL RECORDING Simultaneous recordings were made of potentials from seven electrodes placed at intervals along the muscle. The electrodes consisted of chlorided silver wires mounted in flexible polyethylene tubes filled with sea water. Cotton wicks, about 1 cm. in length, made contact with the muscle. As seen in Fig. 1, the muscle was constantly stretched by a 5 gm. weight suspended from a fine chain, and at the same time was connected to a Grass model FT-02 force-displacement transducer which limited any change of length of the muscle to 2 mm. During electrical recording, the muscle chamber was drained. The electrodes were led to a Grass model III eight channel electroencephalograph. The preamplifiers were alternate coupled, with a time constant of 0.8 sec. A Grass model 2 balance demodulator, in-
installed in the eighth channel, permitted simultaneous recording of tension changes as registered through the force-displacement transducer.

B. ACTIVATION OF THE MUSCLE BY MEANS OF A TRIANGULAR ELECTRODE. In the remaining experiments the muscle was activated by means of a triangular electrode of the type described by Taylor (12, 13). This electrode was used to insure maximal cathodal activation of the muscle, which is possible in the electrical field configuration in this electrode. Evidence will be given elsewhere that this muscle fulfills the necessary requirements for use of the electrode to obtain maximal cathodal activation.

The electrode consisted of a sea water bath with a lateral extension shaped like a right triangle, the side between the 90° corner and the 45° corner opening into the bath, as shown in the center in Fig. 2. Contact with external electrical circuits was made through two silver-silver chloride plates immersed in chambers filled with 3 molar KCl. One such chamber was connected through an agar-sea water-filled channel to the 45° corner opposite the side of the triangle opening into the bath. The other, indifferent electrode, made contact through an agar-sea water-filled channel with the bath at a point 3 cm. from the triangle. Current for stimulation was drawn from a regulated d. c. supply and controlled by a micropositioner relay, the coil of which was driven by a Grass model 4 stimulator. Current pulses of durations as long as 1 sec. or as short as 5 msec. could be passed through the bath. Currents of longer duration could be obtained by activating the relay coil by means of a battery and hand-operated switch. Polarization at the Ag-AgCl electrodes did not alter appreciably the intensity of current through the bath in any of the experiments.

The muscle was placed in the bath parallel to the open face of the triangle and positioned so that one end lay opposite the 90° corner. This is necessary for optimal use of the electrode.

In experiments in which electrical activity was recorded from the surface of the muscle following d. c. stimuli, through the triangular electrode, the electrode was arranged as shown in Fig. 2. The bath portion of the electrode was open at the top. The muscle was held at one end by a clamp fastened to a piece of shell removed from

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**Figure 1.** Bath used in multichannel recording from the surface of the muscle. Sea water is placed in the bath (B). Wick electrodes are shown above the muscle (M) which is stretched by a weight (W) acting over the pulley (P). Tension is registered on a transducer (T) electrically insulated from the bath by insulator I. A rack and pinion mounting (RP) is used to adjust the muscle length.
the animal along with the muscle and, at the other end, by a string attached to a glass bell crank which was, in turn, fastened to a Grass force-displacement transducer (model F-2). Recording electrodes were mounted on the frame which held the muscle attachments and the transducer. The triangular electrode was mounted separately on a rack and pinion so that it could be lowered away from the muscle and recording electrodes during the recording period. Recording electrodes consisted of wicks embedded in agar–sea water in glass tubes with chlorided silver wires as the liquid–metal junction. A traveling probe electrode, used to explore the surface of the muscle, contained no cotton wick but instead consisted of a glass tip drawn out to a diameter of approximately 200 microns. A Grass Instrument Company polygraph (model 5) was used to record both tension and electrical activity. In the latter channel, an EEG preamplifier (model 5P5) having an input time constant of 2 sec. and an amplification of up to 10 µV per cm. was used as routine.

In the experiments in which the mechanical properties of the muscle were exam-
ined, the muscles were placed in a vertically oriented bath of the type diagrammed in Fig. 3. At the bottom, a piece of shell removed from the animal along with the muscle was held in a small lucite clamp at the end of a lucite rod which passed through a rubber finger cot which, in turn, served as a water seal. The lucite rod was attached to a cross-head mounted on a ball-bearing race, so that it could be moved freely upward against one stop for quick release and downward against another for quick stretch. At the top the muscle was attached to a strain gauge transducer (Grass model Ft-02) mounted on a screw adjustment. The bath could be moved with respect to the muscle to bring the triangular electrode into proper position for optimal stimulation (see above). Isometric contractions were used throughout these experiments. Tensions were recorded on a Grass ink writer.

![Diagram of the triangular electrode assembly](image)

**Figure 3.** Schematic diagram of the triangular electrode assembly used to measure the mechanical properties of the ARBM. The electrode is shown at E. The muscle (M) is attached at one end to a force transducer (T) through an electrical insulator (I) and, at the other end, to a cross-head (CH) which moves on ball bearings between two stops (ST). The external electrical circuit is the same as that shown in Fig. 2.

**RESULTS**

**A. MULTICHANNEL RECORDING FROM THE SURFACE OF THE MUSCLE**

When the pedal ganglion and excitatory innervation of the ARBM are intact, numerous small “spontaneous” potentials can be recorded, of 10 to 50 µv. in amplitude and 50 to 100 msec. in duration. These potentials correspond to those described by Hoyle and Lowy (10) except that they are clearly not localized. Simultaneous recording of these potentials from electrodes distributed over the muscle surface (Fig. 4) indicates that the potentials are of maximal amplitude when recorded between the center and one end of the muscle. In recordings between two closely spaced electrodes at either end of the muscle, the potentials are opposite in polarity and are small compared to those recorded from the center. All potentials detected in diphasic channels can be found in the monophasic recordings. No detectable mechanical activity accompanies the potentials shown in Fig. 4.
B. ELECTRICAL ACTIVITY FOLLOWING D. C. STIMULI DELIVERED THROUGH THE TRIANGULAR ELECTRODE  

Stimulation of the ARBM by means of a triangular electrode results in approximately the same pattern of responses to various stimuli as was originally observed by Winton (1). In the triangular electrode, cathodal direct currents produced maximal responses of the d. c. type, whereas anodal and cathodal pulsed currents (30 msec. pulses at 15 pulses per sec.) produce about the same level of isometric contractile tension; however, anodal pulsed currents are more effective in producing quick relaxation. These responses will be analyzed in detail elsewhere.

The experimental procedure used is as follows:—(a) the muscle was stimulated by passing current through the triangular electrode for periods of 1 to
20 sec.; (b) the electrode was then quickly lowered away from the contracted muscle, now in the phase of slow relaxation; (c) the d. c. power supply to the triangular electrode was turned off to avoid a. c. pick-up during the recording period and the EEG amplifier was then switched to "record." This entire procedure, each step of which was necessary before recording could be started, required no more than 20 sec., so that electrical activity, if present in the muscle, could be detected at any time beyond this. This should be ample time, since slow relaxation continued for up to 10 or 20 minutes. In a few of the experiments the muscle was stimulated at a point on its surface by passing current through two of the surface-recording electrodes.

No electrical activity attributable to the muscle was observed during the relaxation period following triangular electrode stimulation (Fig. 5A). In two records out of twenty-one made on nine muscles, small potential changes could be seen during the earliest period of relaxation (Fig. 5B); no further potentials could be seen as the muscles further relaxed, and no discontinuities in the tension records were observed with the disappearance of electrical activity. In these two cases, the muscle had been stimulated by point sources of current (wick electrodes on the surface). In all the other records, no potentials were seen, other than those due to mechanical artifact, which was hard to control but which could be recognized as such.

The muscles used in these experiments were entirely stripped of surrounding tissues during dissection, and would thus correspond to the muscles after removal of the nerve trunks. To test the possibility that the fixed wick electrodes did not detect activity occurring at "active spots," the probe electrode described above was moved along the muscle, and, in some cases, inserted into the body of the muscle. No electrical activity was detected in these experiments. A further check on the recording techniques was obtained by passing a pulse of current through two of the surface electrodes, while recording from two other adjacent electrodes. Electrotonic potentials could clearly be seen at amplifier gains equivalent to 1.5 mV/cm, or approximately 1 per cent of the gain used in the other experiments (Fig. 5E). We would thus conclude that if electrical activity had been present during the post-d. c. relaxation, we should have been able to detect it. In Fig. 5C and D, two experiments are shown in which, prior to stimulation, the muscle had been soaked in sea water containing tetracaine in a concentration of $10^{-4}$ mols per liter. It will be noted that the response to d. c. stimuli has not been altered (compare with record 5B), and that the small potentials which were present following point stimulation in the absence of tetracaine are not present here. It cannot be said with any certainty on the basis of our experiments that tetracaine eliminates the small potentials, since, under normal conditions in this part of the work, potentials appeared only in a few cases. Tetracaine was not tried on preparations in which the ganglia were intact.
Figure 5. Mechanical and electrical responses observed during prolonged relaxation of the ARBM. The mode of stimulation is shown to the left. Triangle (marked STIM) below the muscle indicates stimulation through the triangular electrode and stimulation through wick electrodes is indicated by two arrows marked STIM. Recording electrodes are shown as two arrows marked REC. Tension is given at the top in each record and the concomitantly recorded electrical activity is given below. Dotted vertical line indicates a change of recorder paper speed. The stimulus is delivered at the point marked by an arrow on the tension record. In record E, electrotonic responses to subthreshold pulses (20 msec.) are shown, cathodal responses up from the baseline and anodal responses down. Position of arrows indicating recording electrodes not necessarily that employed in the recording, since (see text) these electrodes were moved along the surface of the muscle.
In the presence of tetracaine, this muscle no longer responds to an anodal pulsed stimulus, as Van Niewenhoven found for faradic stimulation (19). The most effective type of stimulus for eliciting phasic responses in the triangular electrode is the anodal pulsed stimulus. Phasic responses to other stimuli are converted to tonic responses by tetracaine, and the tonic response to a D. C. stimulus is not altered by this drug. If, in this muscle, tetracaine eliminates nerve conduction and perhaps, also, conduction along the muscle cells themselves, then it is difficult to envision a tetanic mechanism for the tonic response which would explain these findings.

C. Quick Release of the Muscle During Relaxation Following Tonic Stimuli. During the period of slow relaxation following contractions due to D. C. stimuli (20 sec. duration), quick release of the muscle to shorter lengths always caused the tension to drop to a new level dependent on the degree of release. A small return of tension toward the prerelease level was observed but the reestablished tension in no case approached the prerelease level. Such small return of tension is commonly observed in rubber or plastic specimens when these are quickly released from a length at which they are under stress. Comparison of the tension return following quick release during post-D. C. relaxation with that seen when release occurs during the stimulus period makes it clear that a qualitative difference exists between these two types of behavior.

These results are illustrated in the recording shown in Fig. 6. The muscle was released from 6 to 10 per cent of its resting length in each case. The muscle was stimulated by cathodal direct currents during the periods indicated by the solid lines below the tension records; quick release occurred at the points indicated by the arrows. In the first record, the muscle was released approximately 1 sec. after each of a series of identical stimuli. The muscle was again stimulated at the new, shorter length. This sequence was repeated several times. The contractile tension decreased as the length of the muscle decreased. Little return of tension followed each release; return would have been expected if the active state were still present.

The ARBM exhibits quick release behavior similar to that shown by tetanized frog skeletal muscles when the release occurs during the stimulus period (Fig. 6B). Quick release immediately following the cessation of the stimulus resulted in little return of tension. Methods used in these experiments did not permit measurement of the time course of decay of the active state; however, these results indicate that the decay is complete within a period of time which is less than 1 per cent of the time required for relaxation of the muscle.

Fig. 6C is a record of the tension recovery following each of a series of quick releases during the stimulus period. It appears that the tension returns to a level determined by the length of the muscle, comparable to the tension elicited by a new stimulus delivered at each shorter length (Fig. 6A).
D. Contractile Responses and Stress Relaxation Following Quick Stretch at Lengths Less Than Rest Length

The experimental equipment in part C was also used in this series. Muscles were stimulated at rest length and at shorter lengths by both pulsed current and cathodal (or anodal) direct currents. Quick stretches were applied following the stimulus in each case to ascertain the extensibility of the muscle. We have made no attempt to analyze

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\text{Figure 6. Experimental records showing tension as a function of time when the ARBM is released to a shorter length during and after direct current (cathodal) stimuli delivered through the triangular electrode. Since consecutive releases were employed in this experiment, the muscle was shortened by quickly turning the screw adjustment on the transducer mounting. Muscles were released from 6 to 10 per cent of rest length at a rate such that the tension dropped 100 ± 12 gm. per sec. Maximum rate of rise of contractile tension in per cent of peak tension was 57 per cent per sec.; tension dropped at from three to five times this rate during quick release of the type employed here. Points at which the muscle was released are indicated by arrows below the records; stimulus periods indicated by horizontal lines. Time intervals, 10 sec.; tension scale to the left, 20 gm. In the absence of quick release following the stimuli, the tension would have decayed slowly, as is shown following the last stimulus in record A.}
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1 The terms used in the quantitative description of the visco-elastic properties of a material are very complex and, in general, involve the postulation of a phenomenological model, such as a Voigt or Maxwell model. Since we have made comparisons on the same muscle and, furthermore, since the complex morphology of the ABRM does not allow simple assumptions regarding the distribution of stresses when the muscle is elongated, we have used the qualitative terms extensibility and stiffness in describing the behavior observed in these experiments. The term extensibility is used in a general way to denote the ability of the muscle to undergo changes in length when a load is applied; a muscle of low extensibility will not elongate to any great extent either initially...
quantitatively the stimulus-induced differences in the time course of stress relaxation following stretch, since visual observation of the stress-time records clearly indicates the relative magnitude of the changes in stiffness appearing under the different experimental conditions. Before each quick stretch the muscle was brought to zero tension and then slowly stretched by a fixed amount (usually 3 per cent of rest length) beyond the length at which tension first appears. This was done to avoid as much as possible errors due to slack within the muscle. The entire procedure was particularly important if comparisons are to be made between the extensibility of the muscle following the stimuli used here, since, in one case, the muscle quickly returns to resting tension and, in the other case, the tension remains high for a considerable period of time. Tension increments due to quick stretch at such high baseline tensions may give errors in estimation of relative stiffness due to such factors as stretch of series elastic elements.

The top record in Fig. 7 shows responses of the ARBM to pulsed and d. c. currents at rest length, followed in each case, by quick stretches of a fixed amount. Since an anodal d. c. stimulus does not reverse the effects of a cathodal stimulus in this muscle, but in all likelihood activates that small portion of the muscle not activated by a cathodal stimulus (see Taylor (12)), an anodal stimulus was delivered before each cathodal stimulus, giving rise to one or two humps on the records. On the top record quick stretch responses indicate that the stiffness following the d. c. stimulus is considerably greater than that following the pulsed current stimuli and that a pulsed current stimulus delivered after the d. c. stimulus reverses the effect of the latter stimulus on muscle stiffness.

In the second record, the muscle was stimulated at 80 per cent of rest length. The first d. c. stimulus (consisting of anodal followed by cathodal current) produced little contractile tension and the ensuing quick stretch indicated little stiffness. The second d. c. stimulus, however, produces slightly more contractile tension, perhaps due to reduced extensibility of the elements in series with the contractile elements. The stiffness following this second
stimulus is virtually as great as that following the d. c. stimulus at rest length. At this length, the muscle shows virtually no stiffness following pulsed current stimuli. The same sort of behavior is seen in the bottom record for the same muscle at 70 per cent of rest length. At the end of the latter record, the muscle was stretched in a series of three stretches back to rest length and a pulsed current stimulus was delivered to the muscle. It is seen that a pulsed current

![Graph showing experimental records.](image)

**Figure 7.** Experimental records, showing tension as a function of time for various stimuli and for quick stretches of constant amount at rest length and at 80 and 70 per cent of initial rest length. Rest length taken as the length at which a reproducible resting tension appears. Maximal tetanic stimuli (20 msec. pulses delivered at 15 per sec.) are indicated by arrows marked T. Maximal direct current stimuli (with triangle first anodal and then cathodal for 15 sec. in each case) are indicated by arrows marked d. c. The muscle was stretched 6.7 per cent of its initial rest length within 80 msec. at points marked S. Before each stretch, the muscle was released to zero tension and brought to a length 3 per cent above the length at which tension reappears. Last series of three stretches represents stretch of the muscle from 70 to 95 per cent of rest length. Time intervals on bottom line one minute; tension indicated to the right 100 gm.
stimulus reverses the increase in stiffness brought about by the D. C. stimulus at 70 per cent of rest length. It would appear that, even at lengths at which the muscle is unable to develop much contractile tension, it still is capable of undergoing a considerable change in extensibility which has a basis similar to that underlying slow relaxation following D. C. stimuli at rest length, since both can be reversed by pulsed current stimuli.

**DISCUSSION**

Observations of small, spike-like potential changes in recordings from the surface of the ARBM when it is slowly relaxing do not necessarily indicate that tension is being maintained during this period by continuous tetanic activation. These potentials are not well correlated with the level of tension maintenance, and can be recorded in the absence of detectable tension. In cases in which the stripped muscle is activated uniformly by cathodal currents over most of its length, as is achieved with the triangular electrode, the small potentials are rarely seen. Since responses to stimuli are similar in every respect to those obtained by Fletcher (2) and Winton (1), it seems unnecessary to postulate a different mechanism for activation of the muscle by means of the triangular electrode. Thus the fact that small potentials are rarely seen in such cases, in addition to the evidence that these potentials are not associated with active tension development, would indicate that the presence of such potentials, and the muscular activation which they might represent, are not necessary for prolongation of relaxation in the ARBM.

The origin of the small potential changes will be discussed elsewhere (Twarog (20)). The distribution of the potentials in multielectrode recordings from the surface of the muscle shows that they are not localized and thus do not represent local spots of ganglionic activity. Typical basophilic ganglion cells are present along the nerve trunks but are not present in the body of the muscle (List (17); Deane and Twarog (18)). The small potentials appear to arise at the point of motor innervation near the center of the muscle and then conduct with a reduction in amplitude to either end of the muscle. This evidence would suggest that these potentials are initiated by nervous activity. In view of their relatively long duration, they appear to represent muscle responses to asynchronous firing of nerve fibers originating from within major nerve trunks.

It has also been shown (Twarog (20)) that when contractions are initiated by stimulation of the nerves supplying the byssus retractor or when spontaneous contractions appear, large, synchronous potential changes are seen, which are similar in amplitude and duration to potentials described as the muscle action potential by Fletcher (14), Prosser et al. (15), and Schmandt and Sleator (16). There is very little to suggest that the asynchronous activity
which the small potential changes probably represent is associated with a
means of muscle activation which, in and of itself, is capable of maintaining
the high tensions achieved by the muscle when activated by d. c. stimuli.

Contractions brought about by direct current stimuli are about as large as
those obtained by stimulation with repetitive currents. The former type of
contraction occurs at the cathode, and, as has been shown by Fletcher (14),
only one large potential change of the synchronous type mentioned above
occurs at the onset of stimulation, followed by a prolonged depolarization
which lasts for the duration of the stimulus. Depolarization also accompanies
contractions brought about by application of acetylcholine (Twarog (21)),
but an action potential does not always precede contraction in the latter case.
In both cases, the membrane potential returns quickly to the resting level fol-
lowing the stimulus; the muscle, however, relaxes very slowly (Twarog (21);
Welsh (22)). From these facts, one would conclude that in its initial phases,
the d. c. contraction is similar to a contracture brought about by continued
membrane depolarization (Sandow (23)). During the period of prolonged
relaxation, the muscle cannot be made to relax more quickly when the mem-
brane is hyperpolarized by, for instance, making the triangular electrode
anodal. Thus the slow decay of tension during relaxation cannot be attributed
to the slow decay of a contracture, since contractures of the type commonly
observed in skeletal muscles are reversed by membrane hyperpolarization. In
the period of post-d. c. relaxation, then, the maintenance of tension cannot
be ascribed to either a tetanic mechanism or a depolarization contracture.

The finding that the active state of the muscle is not maintained beyond the
duration of the stimulus in the case of d. c. stimuli further indicates that a
tetanic mechanism is not operating to maintain tension. During this period,
the muscle behaves like a viscous-elastic body in which the extensibility has
been decreased. The tension drops to zero with only a small release of the
muscle, and, as indicated in our last series of experiments, restretching results
in a large increase in tension which far exceeds that observed when the muscle
is given a similar stretch at rest. It is possible that when ganglia are present,
there is some maintenance of the active state by the larger neuromuscular
barrage. Our quick release experiments were done on stripped muscles which
had been activated through the triangular electrode. In this case, small po-
tentials were not observed, but since the rate of decay of tension in the latter
case is not greater than it is in cases in which small potentials are present, it is
unlikely that the system responsible for these potentials contributes much to
maintenance of an active state and thus the tension.

The fourth series of experiments described in this paper, in which muscle
extensibility was measured at rest length and at lengths less than rest length,
leads support to the first hypothesis described in the introduction, namely,
that certain stimuli bring about changes in the mechanical properties of the
muscle capable of maintaining tension once it has been actively produced, thus prolonging relaxation. That this is possible in systems consisting of fibrous proteins is seen in the behavior of the actomyosin thread. Such threads will contract in the presence of adenosinetriphosphate (ATP) in an appropriate ionic medium. If ATP is then removed from the medium, tension developed during contraction remains high and the fiber is much stiffer than it is in the presence of ATP (Weber (6)). It is thus possible to maintain tension developed during contraction by an increase in stiffness at the peak of contraction, with concomitant removal of the agent responsible for development of contractile tension. While the removal of ATP from the contractile proteins in the intact ARBM is probably not responsible for the maintenance of tension during post-D. C. relaxation, any system in parallel with the contractile system capable of undergoing a change in stiffness would serve the same purpose.

As is shown in Fig. 7, there is a large discrepancy between the ability of the muscle to develop contractile tension and its ability to develop tension in response to quick stretch. This is particularly marked at 70 per cent of rest length. Here the contractile tension developed in response to a maximal stimulus is very small, but the response to stretch approaches that observed following d. c. stimuli at rest length. The tension following stretch at 70 per cent of rest length is considerably above that which would have been observed if the muscle had been brought to this length by pulsed current stimuli rather than d. c. stimuli. The two responses, response to d. c. stimuli (or to any other type of electrical stimulus) and the response to stretch following d. c. stimuli, each show an entirely different dependence on muscle length in this range of lengths. This would be possible if the d. c. stimulus were capable of producing a change in the mechanical properties of the muscle in addition to contraction, perhaps by decrease in the extensibility of a parallel system, but it would hardly be possible if the alteration of the state of the muscle following d. c. stimuli were due to continuation of contractile activity as it is usually conceived. Since the post-d. c. tensile response to stretch is altered by pulsed current stimuli in the same manner as post-d. c. relaxation, it appears likely that both have a common origin. The other evidence presented in this paper points to a change in extensibility of the muscle during this period, so that an increase in the stiffness of a system in parallel with the contractile system is probably responsible for alteration of the relaxation rate. Since these experiments were done at lengths less than equilibrium length, the passive parallel elastic component which appears when the muscle is stretched above equilibrium length is not a complicating factor in our experiments (see Abbott and Lowy (24)).

During the phasic response to tetanic stimuli using interdigitated anodal and cathodal electrodes, Abbott and Lowy (25) have found that the active state, as measured by quick release, is clearly present and that the rate at
which the muscle is released determines the degree of tension redevelopment. Since rates of release of the order of seconds were required to reduce the degree of tension redevelopment to small values, this effect could not account for the absence of tension return during the post-0. c. period in our experiments. Muscles were released in approximately 0.5 sec. Abbott and Lowy have proposed that a certain amount of “set” is present during contractions of the ARBM and that this, being greater than that in skeletal muscles, could account for the greater efficiency of tension maintenance in this muscle. However, relaxation following 0. c. stimuli and acetylcholine application is much slower (by more than two orders of magnitude) than that following tetanic stimuli, thus this effect could not be accounted for by the presence of more pronounced set per unit of contraction unless tension were being maintained by a tetanic mechanism. But since the active state, present while tonus-inducing stimuli are being applied to the muscle, disappears very quickly after the cessation of such stimuli, relaxation could hardly be prolonged by a fusion of individual contractions, each exhibiting pronounced set.

The following scheme can be visualized to explain the prolonged responses of muscles in the intact animal. The task which the so called “catch” muscles must perform is that of maintaining tension against the counterforce exerted by the shell ligaments, in the case of the bivalve adductor muscles, or forces tending to pull the animal away from its rock substratum, in the case of the byssus retractor muscles of Mytilus. If the unit response of the muscle fiber to reflex activation involves both contraction and a change in the extensibility of a system of elements in parallel with the contractile system, then an effective counterforce to forces mentioned above could be developed and maintained with little energy requirement beyond that required to set up the forces initially. Relaxation of the muscle could be controlled by a second set of innervating fibers which alter the state of the parallel catch system, perhaps by secretion of an agent similar to or identical to 5-hydroxytryptamine, which, when applied to intact muscles, eliminates the tonic response. According to this scheme, in the intact muscle contractions of the 0. c. type could be set up by a synchronous volley of impulses from the ganglia. Since single 0. c. contractions in vitro relax at a rate (complete within 20 to 30 minutes in some cases), which could not account for the prolonged contractions encountered in the intact animal, which last from hours to days, reactivation would be necessary in vivo but only at a frequency much lower than would be encountered in a tetanic response involving summed phasic responses, assuming even the slower contraction parameters found for these muscles (Abbott and Lowy (25)). Relaxation could result from the activation of the innervating

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2 While this paper was in the final stages of preparation, a short communication appeared in which Lowy and Millman propose a system similar to that outlined here for prolonged maintenance of
system which reverses the change in extensibility originally appearing with the initial contraction. Lowy has found that records of electrical activity during contractions of intact adductor muscles show a sequence which fits this scheme. In his experiments, initial contraction is accompanied by a burst of impulses which subsides to constant low frequency level maintained during the period in which the shell is closed. Relaxation is accompanied by a second burst of activity.

Dual innervation is common among invertebrates (Wiersma (26)), and in our experience, stimuli which act to depolarize the membrane, such as cathodal currents and acetylcholine, produce the type of response which exhibits prolonged relaxation. Fast relaxation is brought about by different chemical agents (5-hydroxytryptamine) or by stimuli of short duration. In evidence to be presented elsewhere we will show that, in the triangular electrode, trains of short anodal pulses are the most effective in bringing about fast relaxation. Since anodal currents in and of themselves do not produce relaxation during post-D. C. slow relaxation, and short cathodal pulses are capable of producing small responses of the D. C. type, the action of anodal pulses can best be explained by saying that these pulses activate nerve elements which act to produce fast relaxation, or according to our scheme, to reverse the cathodally induced change in extensibility of the fiber. The responses to anodal pulses are not complicated by additional small tonic responses, as would be the case for the responses to cathodal pulses. This explanation of the effect of anodal pulses is borne out by the fact that these responses disappear when the muscle is treated with low concentrations of cocaine or tetracaine, or when excess Mg++ is added to the medium (27). The cathodal D. C. response, on the other hand, is not affected by tetracaine. In

isometric tension (28). On the basis of their findings, these workers postulate that linkages are set up in the "sliding mechanism" of the actomyosin system while the active state is present, and that a second set of linkages, which break at a much slower rate, maintain tension. These second linkages were attributed to changes in the paramyosin fibril system. It was further postulated that a system of inhibitor nerves controls the rate of breakage of the second or "tonic" linkages. Our results lead us to the conclusion that it is essential that a clear distinction be made between the two systems.

In the contractile system, tension is developed or the muscle shortens by a process which is phenomenologically described by Hill's equations and which involves the appearance of the active state. The second system, in parallel with the first, maintains tension by a change in the visco-elastic properties. These properties are observable whether or not tension is present; the muscle is much stiffer during prolonged relaxation. Since the word tonus is usually associated with activity of the contractile system (see reference 27), another term perhaps should be adopted to designate the function of the second, visco-elastic system. As to the changes in the paramyosin fibrils which underlie the increase in muscle stiffness, one of us (WHJ) has suggested, along with other collaborators (9), that the protein paramyosin "crystallizes" within the fibrils, thus forming a closely knit and mechanically rigid system. This suggestion is based on the solubility behavior of the isolated protein and on the behavior of glycerinated byssus retractor muscles. Thus the suggestion of Lowy and Millman may have a demonstrable molecular basis.
the presence of the latter, or excess Mg++, only d. c. type responses can be obtained from the muscle. Cocaine or tetracaine is likely to block nerve fibers and Mg++ may serve as a curarizing agent (see reference 2).

While the above scheme, involving changes in extensibility of a parallel system in addition to those in the contractile system, is only a first approximation, it does fit most of the data presently available on contraction of tonic muscles in molluscs and explains the experimental findings which led early investigators to propose a catch mechanism.

One of us (B. M. T.) wishes to express sincere thanks to Dr. F. A. Quadfasel and Dr. W. J. Friedlander of the Boston Veterans Administration Hospital, who made possible the use of the eight channel encephalograph.

Note Added in Proof.—In a recent paper describing quick release and stretch experiments, B. R. Jewell (J. Physiol., 1959, 149, 154) has presented quantitative evidence for the necessity to differentiate between a state which he has designated the fused state (similar to our catch state) and tetanic contractions of the ARBM. The mechanism proposed by Jewell to explain prolonged contractions of this muscle is similar to that proposed in this paper.

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