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THE CONTRACTILE MECHANISM OF THE ANTERIOR BYSSUS RETRACTOR MUSCLE OF *MYTILUS EDULIS*

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CONTENTS

The mechanical properties of the living, isolated anterior byssus retractor muscle of *Mytilus* (*ABRM*) have been studied under isometric conditions, and during length changes at constant speed. Stimulation of the muscle produces two distinct types of response, characterized by their tension decay rates. The rate is high in a phasic response produced by repetitive stimulation, and low in a tonic response produced by direct current or acetylcholine (*ACh*) stimulation. After cessation of contractile activity (i.e. the ability to shorten and develop tension actively), in both phasic and tonic responses the decay of tension becomes exponential. The time constant of tension decay ranges from 1 to 7 s for the phasic response, and from 5 to 100 min for the tonic response. Application of 5 hydroxytryptamine (5 *HT*) to a tonically contracted muscle converts the tonic tension decay rate to the phasic tension decay rate.

The resistance of the muscle to extension is greater during tonic than during phasic stimulation, but much the same during and after tonic stimulation. This is interpreted to indicate that the system which is responsible for tonic contraction is in action during tonic stimulation. Nevertheless, there is no difference in either the shortening speed at zero load, or the rate of rise of tension

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(after a release) during tonic and phasic stimulation; and this is also true for the muscle's undamped series compliance.

In both phasic and tonic contractions, tension is developed actively from 0·2 to 1·5 l_0, where l_0 is defined as the shortest length of the muscle at which resting tension can be detected. Maximum tension is developed near l_0 and decreases on either side of this optimum. The shape of the isometric tension–length curves is similar for phasic and tonic (*ACh*) contractions, but about 20 % more tension is usually developed in the latter case.

When the unstimulated muscle is slowly extended above l_0 two types of tension are produced: (1) true resting tension, probably due to inert elastic material in parallel with the contractile apparatus, and (2) a variable amount of tension identical to passive tension (i.e. the tension remaining after stimulation when the muscle's ability to shorten and develop tension actively is over). At any particular muscle length, the sum of passive and active tension is nearly the same, although there may be large variations in the amount of passive tension present.

In a twitch, as in a tonic response, tension decays slowly, and greatly outlasts the muscle's ability to shorten and develop tension actively. When the muscle is stimulated with single shocks spaced 10 to 30 s apart, a high level of tension can be developed, and maintained for periods up to 20 h. Such an intermittent activation mechanism may operate *in vivo* during tonic contraction of the smooth retractor and adductor muscles of lamellibranch molluscs, thus enabling tension to be maintained very economically. Tonic tension is, in fact, often maintained in the isolated *ABRM* (stretched above l_0) by 'spontaneous' contractions occurring about once every 10 s. Each individual contraction, which resembles a twitch, is accompanied by one or more action potentials similar to those observed in twitches, or at the onset of repetitive stimulation.

It is suggested that tension in the *ABRM* is developed and maintained by a system similar to that in vertebrate striated muscle, the *ABRM* system being specialized in that under certain conditions tension decays extremely slowly. A hypothesis in terms of a sliding filament contractile mechanism is put forward which postulates: (1) that contraction in the *ABRM* is due to linkages formed between two kinds of filaments, and (2) that the breaking rate of these linkages (and thus the rate of tension decay) is governed by the concentration of a relaxant (probably 5 *HT*), the single process of breaking of linkages being reflected by the exponential phase of tension decay. The results are interpreted on this hypothesis, and discussed with reference to another hypothesis (Johnson, Kahn & Szent-Györgyi 1959; Rüegg 1959, 1961*a*), which holds that in addition to a contractile (actomyosin) system, muscles like the *ABRM* have a second (paramyosin) system which becomes rigid during tonic contraction, and thus maintains the tension developed by the contractile system.

1. Introduction

On the sliding filament hypothesis, contraction in vertebrate striated muscles can be considered in molecular terms (Hanson & Huxley 1955; A. F. Huxley 1957). This is not possible at present for any other type of muscle. In the case of certain molluscan smooth muscles, however, much new information has accumulated in recent years so that their contractile mechanism can now be discussed in terms not far above the molecular level.

In such molluscan muscles the contractile apparatus, like that of vertebrate striated muscles, consists of two kinds of discontinuous filaments. In both types of muscle one kind of filament is thin, with a diameter of about 50 Å. The other kind is thicker, and over certain regions bears regularly-spaced structures (bridges), which project at right angles to the long axis of the filament (Huxley 1957; Hanson & Lowy 1959, 1961; Lowy & Hanson 1962). But, whereas in vertebrate striated muscle the thick filaments have a diameter of about 100 Å, the thick filaments in the molluscan muscles can have a diameter up to 1500 Å (Hodge, Huxley & Spiro 1954; Hanson & Lowy 1957). Longitudinal sections of the thick filaments show an internal structure similar to that seen in isolated filaments (Hanson, Lowy, Huxley, Bailey, Kay & Rüegg 1957; Elliott, Hanson & Lowy 1957;

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Elliott 1960), where it was given the name paramyosin (Hall, Jakus & Schmitt 1945). This last-mentioned structural feature is due to the presence of tropomyosin A (Hanson *et al.* 1957; Elliott *et al.* 1957), a protein without adenosine triphosphatase (*ATP*-ase) activity, which does not combine with actin (Rüegg 1957). In these molluscan muscles there can be at least twice as much tropomyosin A as actomyosin (Rüegg 1961 *a*). Johnson, Kahn & Szent-Györgyi (1959) use the term paramyosin to describe the protein tropomyosin A.

In the psoas muscle of the rabbit the thin filaments are about 1 μ long and they contain all the actin; the thick filaments are about 1·5 μ long and their principal constituent protein is myosin (see Huxley & Hanson 1960). Information about the composition of the filaments in the contractile apparatus of the molluscan muscles is not nearly so complete. But there is evidence that, in comparison with the rabbit muscle, both kinds of filament can be more than ten times as long, that all the actin is present in the thin filaments and tropomyosin A in the thick filaments, and that the thick filaments may be composed of a sheath of myosin surrounding a core of tropomyosin A (Hanson & Lowy 1960, 1961, 1962; Lowy & Hanson 1962).

Two physiological features of lamellibranch smooth muscles are so unusual that they have been studied intensively for many years. These are the ability to maintain a high level of tension for long periods without fatigue, and the presence of a mechanism which specifically controls the relaxation phase of the contractile cycle. The muscle which has been used for most of the recent experimental work is the anterior byssus retractor muscle of *Mytilus* (*ABRM*). Winton (1937) showed that direct current (d.c.) stimulation of the *ABRM* produces a contraction in which relaxation may not be complete for several hours. He also discovered that 50 c/s alternating current (a.c.) applied to such a slowly relaxing muscle causes complete relaxation in less than a minute. Furthermore, Winton found that a.c. applied to a completely relaxed muscle produced a contraction in which relaxation is complete in less than a minute. We shall call such a response a *phasic contraction*, and the stimulation which produces it *phasic stimulation* (figure 1 *a*). More recently Twarog (1954) observed that application of acetylcholine (*ACh*) to the *ABRM* gives a slowly relaxing response (figure 1 *b*, *c*), which closely resembles that obtained by stimulation with d.c. We shall call such a response a *tonic contraction*, and the two types of stimulation which produce it (d.c. and *ACh*) *tonic stimulation* (figure 1 *b*, *c*). Twarog also showed that application of 5 hydroxytryptamine (5 *HT*) to a slowly relaxing muscle causes relaxation which can be complete in less than a minute, i.e. 5 *HT* produces an effect like that obtained with a.c. under the same conditions. The 5 *HT*-treated muscle still responds to *ACh* (Twarog 1954), and to d.c. or a.c. (Hoyle & Lowy 1956), but the contraction in all cases is a phasic one. Lowy & Millman (1959 *b*, *c*) produced evidence that 5 *HT* specifically affects the relaxation phase of the contractile cycle. We therefore propose to call 5 *HT* a relaxant.

From experiments with the smooth part of the adductor muscle of *Pecten*, Bozler (1930) deduced that relaxation can be speeded up by activation of 'inhibitory' nerves. In the *ABRM*, there is good physiological evidence for the presence of such nerves (van Nieuwenhoven 1947; Hoyle & Lowy 1956; Takahashi 1960), and it has been suggested that their activation leads to the release of relaxant (Lowy & Millman 1959 *b*, *c*).

Tonic contraction has been explained hitherto on two opposing hypotheses. According to the catch mechanism hypothesis (e.g. von Uexküll 1912; van Nieuwenhoven 1947),

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certain elements in the muscle 'set' or 'catch' in the shortened state, and tension is thus maintained passively. On the tetanus hypothesis (e.g. Bayliss 1928; Hoyle & Lowy 1956), tension maintenance is due to continuous activity—a mechanism analogous to a tetanus in vertebrate skeletal muscle.

Recent physiological experiments (Lowy & Millman 1959*a*, *b*, *c*; Jewell 1959*a*, *b*; Johnson & Twarog 1960) have shown that tonic contraction in the *ABRM* cannot be explained fully by either the catch or the tetanus hypothesis as they stand (see Jewell 1959*b*). These experiments show that although tension is maintained by continuous activity during tonic and phasic stimulation, a slowly decaying tension (tonic tension)

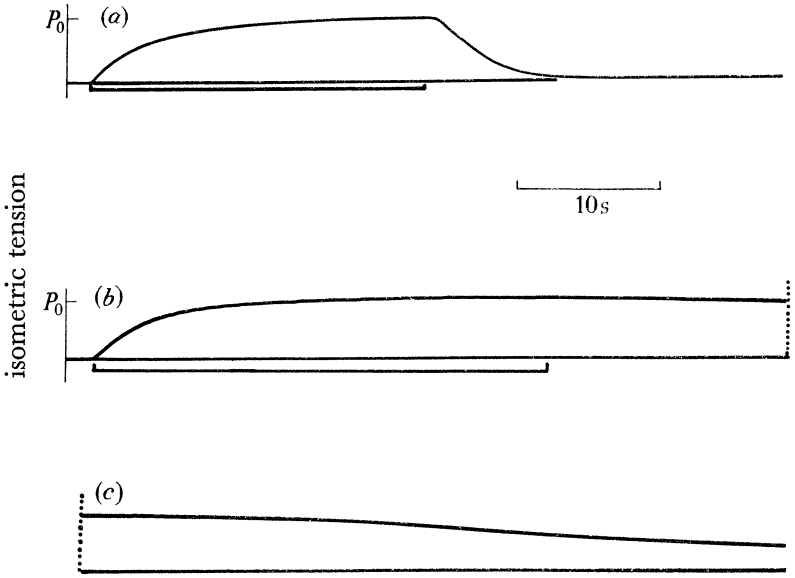

FIGURE 1. Isometric responses of the *ABRM* of *Mytilus*. (*a*) Phasic contraction produced by repetitive stimulation at a frequency of ten shocks per second; (*b*) tonic contraction produced by 10^{-5} g/ml. *ACh*; (*c*) continuation of (*b*). The period of stimulation is indicated below each record by a solid line. Temperature = 22 °C.

remains after tonic stimulation, when the muscle shows the inert behaviour of a viscous-elastic body, i.e. tonic tension is not caused by continuous activity (Jewell 1959*b*; Johnson & Twarog 1960). But tonic tension decays slowly, and therefore to *maintain* tonic tension periodic re-excitation is necessary, though at a frequency much lower than that required to maintain tension during a phasic contraction.

The above results can be considered in terms of assumptions essentially similar to those proposed by Bozler (1930) to account for contraction and relaxation in the *Pecten* muscle. Thus it has been argued (Lowy & Millman 1959*b*, *c*; Jewell 1959*b*; Johnson & Twarog 1960; Takahashi 1960) that in the *ABRM* there exist two control systems: one excitatory, the other 'inhibitory', the latter being responsible for the relaxant effect. When the two systems are activated together relaxation is fast, giving a phasic contraction. Activation of the excitatory system alone gives a tonic contraction, while rapid decay of tonic tension can be brought about by activation of the 'inhibitory' system.

The problem of relating structure and function in muscles like the *ABRM* has recently been approached in two ways. One is based on studies of the properties of paramyosin (tropomyosin A), and on mechanical experiments with glycerol-extracted preparations of

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the *ABRM* and similar muscles in the presence of agents which are known to exert certain effects on similar preparations of rabbit psoas muscle. The evidence thus obtained has led Johnson *et al.* (1959) and Rüegg (1959, 1961 *a*, *b*) to put forward a hypothesis to account for the behaviour of living molluscan muscles. According to these investigators, the muscle fibres contain two independent systems acting in parallel, tension being developed in an actomyosin system and then maintained by some change of state of the protein in a paramyosin system. We propose to call this the *independent catch hypothesis*. It assumes that 'the tension developed by the actomyosin system may be preserved by the paramyosin system for an indefinite time without any further active processes and without the need for a continuous expenditure of energy' (Johnson *et al.* 1959, p. 161). This does not mean, however, that *in vivo*, periodic reactivation is unnecessary (see Johnson & Twarog 1960, p. 957).

In the other approach most of the evidence comes from structural work (Hanson & Lowy 1959, 1960, 1961; Lowy & Hanson 1962), and from experiments with living muscles (Lowy & Millman 1959 *a*, *b*, *c*). The results obtained suggest that in muscles like the *ABRM* tension is both generated and maintained (as in vertebrate striated muscle) by means of linkages (forces of some kind) between thick and thin filaments in a sliding filament contractile system. For the molluscan muscles we assume that the rate at which these linkages detach during isometric relaxation is controlled by a relaxant (e.g. 5 *HT*). When the relaxant is absent the linkages detach extremely slowly, and as a result the decay of tension can be two orders of magnitude slower than decay of contractile activity (i.e. the muscle's ability to shorten and develop tension actively). This situation corresponds to a tonic contraction. Tension can be maintained economically because the linkages have to be renewed by activation only at infrequent intervals. When the relaxant is present at a concentration sufficient to affect all the reactant sites, the linkages break at a relatively high rate and the muscle gives a phasic contraction. This explanation of the contractile mechanism in lamellibranch smooth muscles will be called the *linkage hypothesis*.

This hypothesis focuses attention on the processes by which the relaxant exerts its action. No comparable relaxing mechanism has yet been discovered in other contractile systems. In terms of the sliding filament hypothesis, our view is that the relaxant does not affect the rate at which linkages are formed, but specifically controls the reactions that govern the rate at which linkages detach. Such a mechanism would represent an important specialization of the contractile process which, however, could still be considered as basically similar to that of vertebrate striated muscles.

The main object of this paper is to present the results from physiological experiments with the *ABRM* on which the linkage hypothesis is based.

Some of the results presented here have been published previously in preliminary communications (Lowy & Millman 1959 *a*, *b*, *c*).

2. Methods

Mussels (*Mytilus edulis*) were obtained from Plymouth and stored in tanks of aerated Plymouth sea water. The *ABRM* was dissected as follows.

The animal was opened by cutting the adductor muscles. The byssus, foot, and soft organs were removed, leaving the retractor muscles. A platinum hook was tied firmly to

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the byssal mass by means of a soft cotton thread. At the shell end of one of the anterior retractor muscles, a small piece of shell at the muscle's insertion was chipped free on three sides but left attached to the hinge on the fourth side. Two small holes were drilled in this piece of shell, and a platinum connector was wired to it. The other retractor muscles were cut free from the preparation, and the shell attachment was chipped off at the hinge.

In two series of experiments (see figures 12 and 16) a slightly different preparation was used. The byssus end of the muscle was tied (with cotton thread) to a piece of heavy gauge platinum wire, and the shell was screwed to the bottom of the chamber. This reduced the series compliance of the apparatus to about one-third of that in the usual preparations (cf. figure 12*b*).

The isolated muscle was suspended in aerated sea water under a light load (10 g) for a period of two hours or more before being used for experiments. The muscles were of length (l_0, see §3(*i*)) 1·8 to 3·3 cm (average = 2·7 cm), and weight 10 to 65 mg (average = 37 mg) (values from the 60 muscles described in table 1*a*).

For the experiments the muscle was suspended vertically in a chamber which could be used in two ways. In one, the chamber was filled with aerated sea water, which was run out during electrical stimulation. In the other, a steady flow of sea water ran over the muscle from a container above. The advantage of the latter method was that the muscle could be stimulated without being exposed to air, but had the disadvantage that slight fluctuations in the flow of sea water were sometimes perceptible on the tension record. The constant-flow method was chiefly used for the experiments involving electrical stimulation for long periods, the recording of electrical muscle activity, or the rapid washing of *ACh* from the muscle.

All experiments were performed at room temperature (18 to 25 °C, average = 21 °C). In some experiments artificial sea water prepared according to Hodgkin & Katz (1949) was used, but no difference was detected between these experiments and those in which natural sea water was used.

In all experiments, except those involving electrical recording, the platinum connexions at either end of the muscle were used as stimulating electrodes, thus producing a longitudinal current through the muscle. In the experiments during which electrical activity was recorded, the two stimulating electrodes (separated by 5 to 10 mm) were placed in contact with the muscle at one end. Unidirectional square-wave pulses of variable duration, voltage, and frequency were provided by a single channel electronic stimulator. Repetitive stimulation produced a phasic contraction which had the same appearance as that produced by alternating current (Winton 1937), or by alternating condenser discharges (Jewell 1960). Direct current stimulation was obtained from a 12 volt dry cell. Reversal of the polarity of the repetitive shocks had no effect on the contraction. Reversal of the polarity of d.c. stimulation, however, often produced greater (or less) tension, but this effect could not be related consistently to the direction of current flow in the muscle.

5 *HT* and *ACh* were made up to a concentration of 10^{-3} g/ml. in distilled water, and phosphate buffer (Pilgrim 1954) respectively, and stored in a refrigerator. Immediately before the experiment they were diluted in sea water to the required concentration. (When *ACh* or 5 *HT* was required in concentrations greater than 10^{-5} g/ml. it was dissolved directly in sea water.) *ACh* was usually applied to the muscle through a pipette, and

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washed off after about 1 min. The resulting contraction was uniform along the length of the muscle, so far as could be detected by visual observation. 5 *HT* was added to the bathing solution to give the required concentration.

Electrical activity was recorded by a pair of cotton threads, soaked in sea water, and tied loosely around the muscle (about 1 cm apart) at the end of the muscle remote from the stimulating electrodes. One end of each thread was then connected through a platinum-platinum chloride electrode to the input of a d.c. pre-amplifier, the output of which was fed to the A_1 channel of a Cossor 1049 oscilloscope. This apparatus could detect electrical signals of 10 μV and above, with a rise time of 0·3 ms (95 % complete).

Constant speed stretches and releases were applied to the muscle by means of a Levin–Wyman ergometer constructed by C. F. Palmer and Co. Length changes were recorded by means of a vane (attached to the arm of the ergometer), which interrupted a light beam directed onto one section of a double photocell. The period between the stretch or release, and the beginning or end of stimulation could be regulated by means of a mechanical timing device.

Tension was recorded by a RCA 5734 transducer valve. Because of the high tensions developed by the *ABRM* it was necessary to connect the muscle to a lever (2 cm long) which reduced the tension transmitted to the transducer by a factor variable from 10 to 1. In this way tensions up to 400 g could be recorded without damaging the transducer. At the most sensitive setting of the transducer, tensions of about 2 mg could be detected.

The transducer output, together with that of the photocell or the d.c. pre-amplifier, was displayed on a Cossor 1049 oscilloscope, and recorded on 35 mm photographic paper.

Before each experiment the muscle was stimulated several times with repetitive shocks until the response was consistent. Reference length (l_0) was determined (see § 3 (*i*)), as well as the threshold for contraction with single shocks of 2 ms duration. The muscle was then allowed to rest for 5 to 10 min before the start of the experiment. In general, a similar rest was allowed after each contraction during the experiment. Throughout each experiment the peak tension, and the rates of rise and fall of tension in a phasic contraction were compared, and if these parameters changed by more than about 20 % the muscle was discarded.

In the 'active-state' experiments, the time of release was varied in 'random' fashion with respect to the stimulus or end of stimulation. In comparing contractions at different muscle lengths, contractions were observed at various lengths, in order, from l_0 to the shortest (or longest) length, and then at the same lengths in the reverse order. These results were averaged for each length. A similar procedure was followed for most other experiments (e.g. those involving stretches); i.e. the various contractions were first produced in a fixed order, then in the reversed order, and the results for each of the conditions were averaged.

The compliance of the undamped series elastic component and the shortening speed of the muscle at zero load were recorded by the following method (Millman 1963). During a maintained tension plateau produced by phasic or tonic stimulation, the muscle was released at a high constant speed (4 cm/s) by about 10 % l_0, an amount considerably greater than that required to drop the tension held by the undamped series elastic component to zero. From the records of tension and length during the release, the stress–strain

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curve of the muscle's undamped series elasticity was determined (see Hill 1950). The time that the muscle had been shortening at zero load could be measured from the tension records; and by subtracting the length change required to drop tension in the undamped series elastic component to zero from the amount of the release, the length shortened at zero load was obtained. From these two measurements, the shortening speed at zero load was determined. Using this method it was possible to measure, *during a single (phasic or tonic) contraction*, not only the shortening speed at zero load and the compliance of the undamped series elastic component, but also the peak isometric tension, and the rate at which this tension is developed (see table 2, p. 124).

3. Results

(*a*) *The isometric twitch response*

Stimulation of the *ABRM* with a single electrical shock of voltage × duration less than ten times threshold, gives a twitch response which is accompanied by a single muscle action potential (Fletcher 1937*b*; Schmandt & Sleator 1955; and figures 4*a*, 5*a*). The threshold voltage for contraction increases with decreasing stimulus duration in much the same manner as has been described for the translucent adductor muscle of *Pinna* (Abbott & Lowy 1956). Typical strength–duration curves of the stimulation threshold for the action potential and for tension development are shown in figure 2. These curves are very similar; the differences are probably due to differences in the sensitivity of the two types of recording apparatus.

When either stimulus voltage, or stimulus duration is increased, the peak twitch tension increases but no well-defined maximum is reached (Schmandt & Sleator 1955). With our method of stimulation we have found that there is little increase in isometric twitch tension when the stimulus strength is increased above four times threshold value. Much stronger shocks (e.g. of 20 ms duration) often produce a contraction larger than the twitch response which is, however, due to repetitive muscle responses, as can be seen when tension and muscle action potentials are recorded simultaneously. In all the experiments described below the shocks used were of 2 ms duration and of 5 to 6 times threshold voltage.

Abbott & Lowy (1958*a*) have shown that in the *ABRM* (at 14 °C), twitch tension reaches a peak of about 0·1 P_0 in 1 to 1·5 s, and decays to half-value in 14 to 16 s. But we find that the latter parameter and peak twitch tension are changed following repetitive stimulation. This is shown in table 1*a*, which gives the characteristics of twitches obtained under two conditions: (1) at least 5 min after any previous contraction (normal twitch), and (2) 1 min after repetitive stimulation. In a normal twitch, the muscle develops a tension of about 0·1 P_0. *We define P_0 as the maximum tension the muscle can develop at l_0 in response to repetitive stimulation (see* §3(*d*)*) and l_0 as the shortest length of the muscle at which a definite resting tension can be detected* (see §3(*i*)). Twitch tension reaches a peak in about 2 s, but decays relatively slowly (table 1).

The curve for the fall of isometric twitch tension always has the same general shape, and for this reason an indication of the decay rate in any particular twitch response is provided by the time taken for tension to decay from peak to half-peak value: this time will be called the *half-peak decay time*. In experiments with a large number of muscles the

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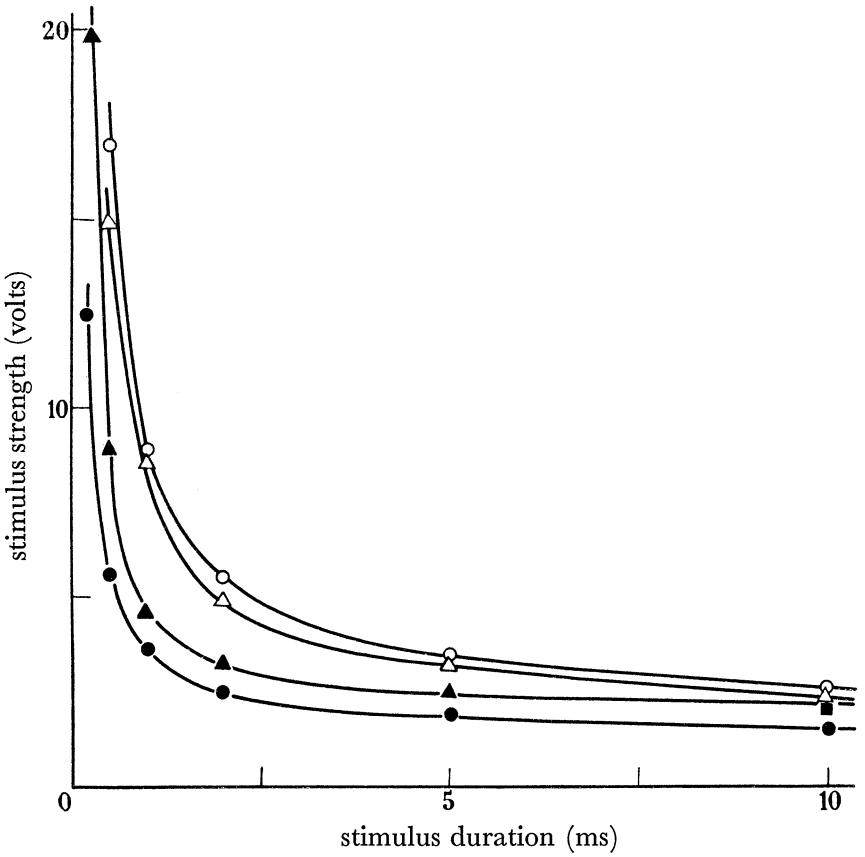

FIGURE 2. Stimulus strength–duration curves for the *ABRM* of *Mytilus*. Thresholds for responses to single stimuli: for isometric contraction (open symbols), and for muscle action potential (solid symbols). Contraction threshold in sea water ○, in sea water with 10^{-6} g/ml. 5 *HT* △; action potential threshold in sea water ●, in sea water with 10^{-6} g/ml. 5 *HT* ▲. The muscle was stimulated at one end by a pair of platinum electrodes, spaced 3 mm apart. Points were averaged from experiments with two muscles; each muscle was 2·8 cm long, and weighed 23 mg. Temperature = 22 °C.

TABLE 1. THE ISOMETRIC TWITCH RESPONSE OF THE *ABRM* OF *MYTILUS*

Temperature 18 to 25 °C, average = 21 °C.

	peak tension (% of P_0)	rise time (s)	half-peak decay time (s)	number of muscles
	a			
normal twitch*	10·6 (4·7†) 2·6 to 20·4‡	1·92 (0·41) 1·2 to 2·8	33 (24) 5·5 to 101	60
twitch, 1 min after repetitive stimulation	17·3 (5·1) 6·4 to 29·7	1·85 (0·29) 1·1 to 2·5	14·0 (7·5) 3·7 to 47	
	b			
normal twitch*	10·3 (4·9) 2·6 to 16·9	1·83 (0·32) 1·2 to 2·2	37 (29) 11·1 to 96	13
twitch, 1 min after repetitive stimulation	16·9 (6·1) 8·5 to 24·4	1·82 (0·23) 1·4 to 2·2	15·1 (7·1) 6·4 to 27·6	
twitch, in the presence of 5 *HT* (10^{-5} to 10^{-6} g/ml.)	11·6 (6·4) 3·5 to 23·2	1·55 (0·30) 1·2 to 2·1	3·3 (1·1) 1·8 to 5·5	

* A twitch produced at least five minutes after any previous contraction.
† Standard deviation of a single observation.
‡ Range of observations.

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half-peak decay time was found to range from 5·5 to 101 s, with an average of 33 s (table 1). (For any particular muscle, the range of values for half-peak decay time is, however, considerably smaller). In contrast, the time taken for tension to rise to peak value (rise time) and the peak tension produced do not vary so much (table 1). The large variation in the half-peak decay time for different muscles is indicated by the wider range of values and the higher standard deviation as compared with the corresponding figures for rise time and peak tension (table 1).

Plots of the logarithm of tension against time (linear) show that as in the frog's sartorius muscle (Jewell & Wilkie 1960), the latter part of the tension decay curve is exponential (figure 3). Values for the time constant of the exponential part of the curve in the *ABRM* range from 5 to 20 min.

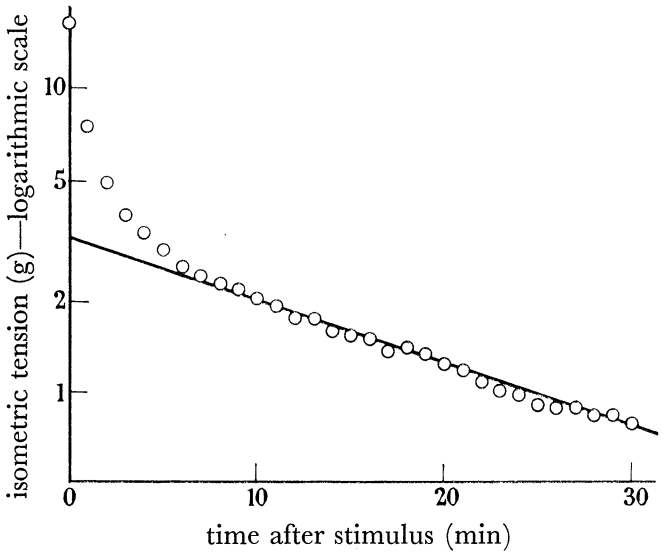

FIGURE 3. Decay of isometric twitch tension in the *ABRM* of *Mytilus*. Time constant of exponential part of tension decay = 21 min. Temperature = 19 °C, length = 2·3 cm, weight = 38 mg.

If the muscle is stimulated by a single shock given less than 2 min after an isometric phasic contraction, more twitch tension is produced than in the normal twitch (i.e. a twitch produced at least 5 min after any previous contraction). As compared with the normal twitch, rise time is not greatly affected, but the half-peak decay time is reduced by a factor of more than 2 (table 1). As in the normal twitch, we find that the latter part of the tension decay curve is exponential. However, the time constant of the exponential part of this curve remains the same, regardless of the time between the stimulus and any previous contraction.

(*b*) *Decay of 'active-state'*

Using the isometric quick-release method of Ritchie (1954*b*), Abbott & Lowy (1958*a*) showed that in the pedal retractor muscle of *Mytilus*, 'active-state' decays more rapidly than tension. We have found this in the *ABRM* as well (Lowy & Millman 1959*a*). Twitches, reproducible within 10% as regards peak tension and time course can be obtained in the *ABRM* if the stimulus is given a short fixed time (e.g. 1 to 2 min) after an isometric phasic contraction. In such twitches we find that the decay of 'active-state' can

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be two orders of magnitude faster than the decay of tension (figure 4). We use the term *passive tension* to describe the tension present, after stimulation, when the ability to shorten and develop tension actively (*contractile activity*) is over.

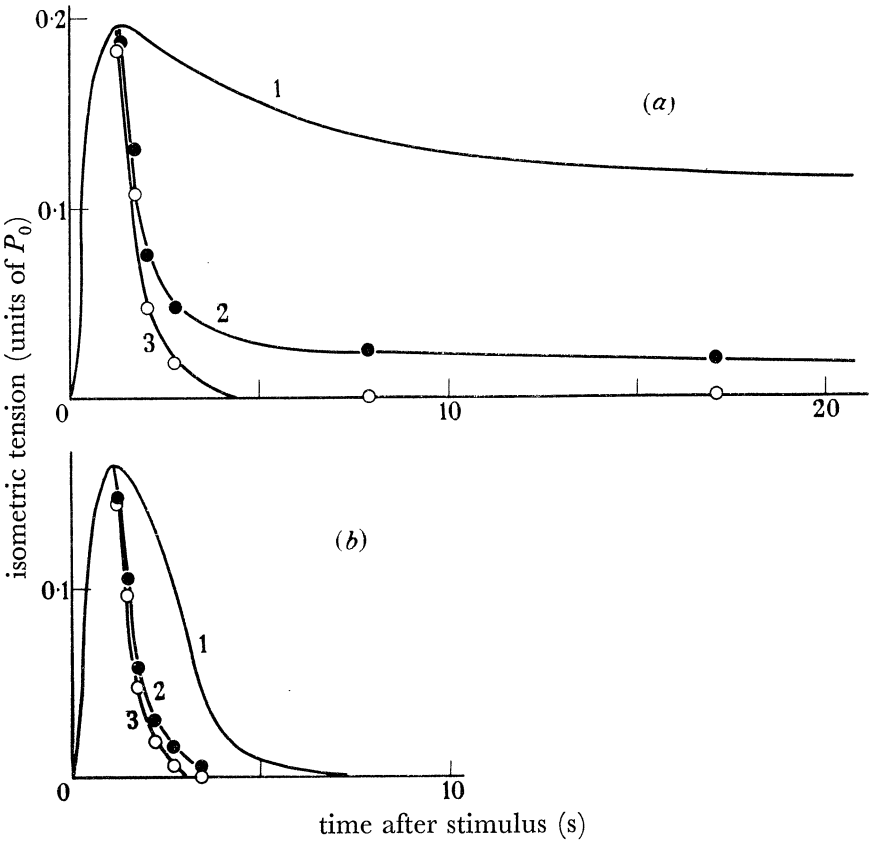

FIGURE 4. Responses of the *ABRM* of *Mytilus* to single stimuli: (*a*) in sea water, (*b*) in sea water with 10^{-5} g/ml. 5 *HT*. Curves (1): isometric twitch. Curves (2) (●): 'active-state', determined by measuring the tension redeveloped following releases of 0·04 cm at a speed of 1·0 cm/s at different times after the stimulus. The points were obtained in a 'random' sequence. Curves (3) (○): decay of contractile activity, determined by subtracting from curves (2) an amount of tension equal to 0·14 of the twitch tension held at the time of release. For details see §4(*f*). Temperature = 24 °C, length = 2·3 cm, weight = 20 mg, P_0 = 5·7 kg/cm^2.

In agreement with other investigators (Jewell 1959*b*; Johnson & Twarog 1960), we have previously shown (Lowy & Millman 1959*a*) that as long as the *ABRM* is holding tension—often several minutes after stimulation—a quick release in which tension drops to zero is always followed by a small recovery of tension. Furthermore, we have found that some time after the stimulus (5 to 10 s in the case of the twitch response), the amount of tension recovered becomes approximately proportional to the amount of tension released (e.g. figure 4*a*). This might suggest that since tension is decaying very slowly, 'active-state' continues at a low and almost constant level for a long time after the stimulus (see figure 4*a*). It seems unlikely, however, that contractile activity would follow such a time course. Jewell found that after *ACh* stimulation 'the muscle exhibits the inert behaviour of a damped elastic body' (Jewell 1959*b*, p. 164). We similarly interpret the proportionality we have observed between the tension released and the tension recovered to indicate the

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presence of passive damped elasticity in the muscle, in series with the usual undamped series elastic component. Thus, a few seconds after a single stimulus, the tension held is passive tension.

There seemed, however, to be a possibility that at least part of the tension recovered might be due to spontaneous contractile activity initiated by the release itself. We have observed that when a muscle stretched above l_0 is released, spontaneous contractile activity associated with action potentials may occur, particularly if the release is very slow. However, all the 'active-state' experiments were performed at muscle lengths below l_0, and the tension recovery records never indicated any spontaneous contractile activity. As a further check, tension and electrical activity were recorded concurrently during some of the release experiments, and no electrical activity that could be associated with the release was observed (figure 5).

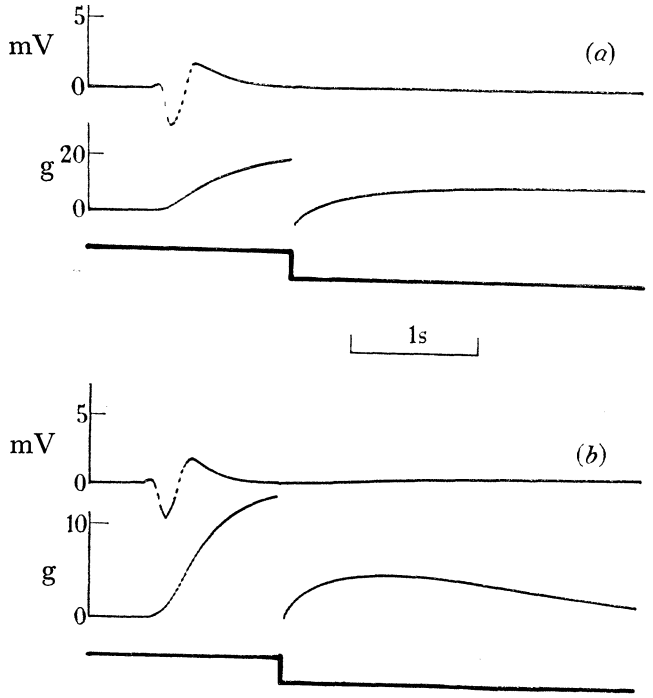

FIGURE 5. Quick release during an isometric twitch of the *ABRM* of *Mytilus*. Electrical activity shown on upper traces, tension on middle traces, and length on lower traces. Muscle stimulated at 2·7 cm, released 0·5 cm at a speed of 1·0 cm/s: (*a*) in sea water, (*b*) in sea water with 10^{-6} g/ml. 5 *HT*. Temperature = 24 °C, l_0 = 2·7 cm, weight = 44 mg. (The small irregularity in the electrical records which appears during the release is caused by the movement of the muscle.)

Experiments were also carried out to show the effects of variations in the extent and speed of release on the amount of tension recovered after a release. Varying the extent of release, it was found that the amount of tension recovered is approximately proportional to the amount of tension released, when the extent of release is such that the tension does not completely drop to zero. For extents of release greater than that required to drop tension to zero, the amount of tension recovered decreases as the extent of the release is increased. The largest recovery of tension is obtained for a release just sufficient to drop tension in the muscle to zero. With different speeds of release, in the range 0·01 to 4·0 cm/s, there is little variation in the amount of tension recovered, although at the slowest speeds

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the amount of tension recovered is slightly reduced. Therefore, in all the experiments described here, the 'active-state' curves were determined using releases of an extent just sufficient to drop peak tension to zero and at speeds of 1·0 or 4·0 cm/s.

(*c*) *The effects of* 5 *HT*

Treatment of the muscle with 5 *HT* (10^{-5} to 10^{-6} g/ml.) does not greatly alter the threshold for the action potential or for tension development (figure 2), but it dramatically affects the form of the twitch response (figure 4). The greatest effect is on the time course of tension decay which is considerably accelerated. Rise time and peak tension are somewhat reduced, but these effects are small compared with the change in half-peak decay time (table 1). As in the untreated muscle, the latter part of the twitch tension decay curve is exponential (figure 6), the time constant being 1 to 4 s as compared with 5 to 20 min in the untreated muscle.

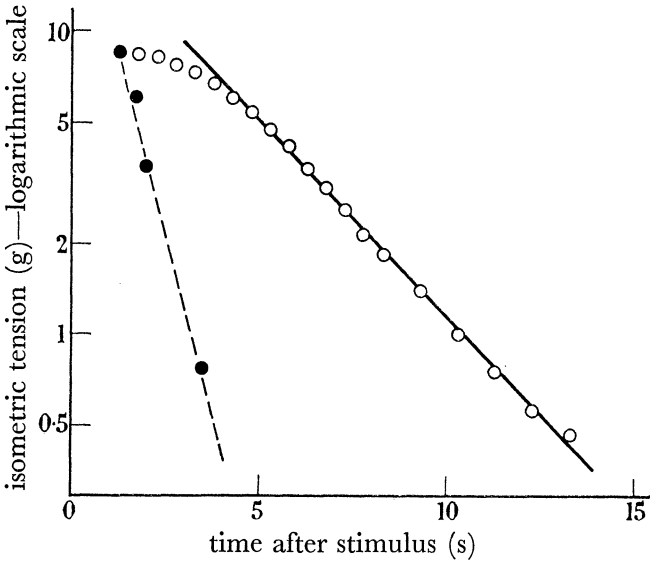

FIGURE 6. Decay of isometric twitch tension (○) in the *ABRM* of *Mytilus* in sea water containing 10^{-6} g/ml. 5 *HT*. 'Active-state' (●) determined by measuring the tension redeveloped after releases of 0·05 cm at a speed of 1·0 cm/s, at different times after the stimulus. Time constant of exponential part of twitch tension decay = 3·4 s. Temperature = 24 °C, length = 3·0 cm, weight = 44 mg.

Although 5 *HT* greatly accelerates the decay of twitch tension, the time course of the decay of 'active-state' is little changed (figure 4*b*). The whole of the 'active-state' curve is shifted slightly to the left, a fact which suggests that 5 *HT* reduces the amount of contractile activity produced in response to a single shock. This explanation could account for the reduced twitch tension obtained in a muscle treated with 5 *HT* (table 1). However, it is shown in table 2 that 5 *HT* does not affect the shortening speed at zero load or the compliance of the series elastic component in the *fully-activated* muscle.

(*d*) *The phasic response*

Repetitive shocks of the same strength as those used for twitches (duration: 2 ms, and voltage: 5 to 6 times threshold) applied at a frequency of 4 to 10/s produce a phasic contraction (figure 1*a*). With the apparatus used, tensions as small as 10^{-4} of the peak tension

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can be detected. At this sensitivity no regular fluctuations in tension can be detected at a frequency above about 4 shocks per second. We have therefore taken 4 shocks per second as the fusion frequency for the phasic response.

We define P_0 as the maximum tension the muscle can develop at l_0 (see §3(*i*)) in response to repetitive stimulation. In response to such stimulation, tensions as high as 11 kg/cm^2 were developed; this is five to ten times that produced by a single shock. Using a multi-electrode assembly, and alternating condenser discharges at 10/s, Jewell (1959*b*) obtained tensions of similar magnitude. The response of the *ABRM* to repetitive stimulation differs from a tetanus in the frog's sartorius muscle in two ways. Firstly, in the *ABRM*

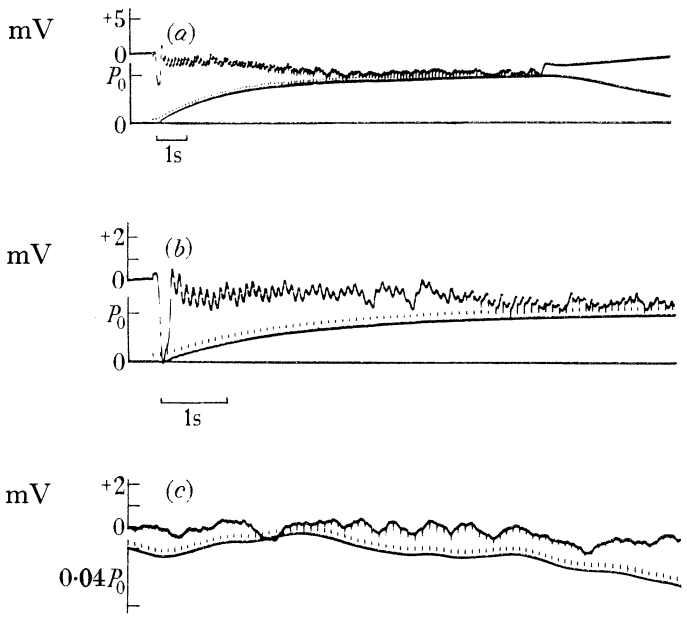

FIGURE 7. Responses of the *ABRM* of *Mytilus* to repetitive stimulation at a frequency of 10 shocks per second. Tension (lower traces) and electrical activity (upper traces) recorded simultaneously. Stimuli are indicated by 'break-through' artifacts on the tension traces. (*a*) Tension and electrical activity recorded at low amplification; (*b*) early part of the response recorded on an extended time scale and at a higher amplification for the electrical activity; (*c*) same response as (*b*), after 27 s of stimulation, using high amplification for the recording of both tension and electrical activity. Temperature = 24 °C, length = 2·7 cm, weight = 44 mg, P_0 = 6·5 kg/cm^2.

the action potentials fuse to give a level of depolarization which disappears as soon as stimulation stops (Fletcher 1937*a*; and figure 7*a*). Secondly, about 20 s after the beginning of stimulation in the *ABRM*, when a plateau of tension has usually been reached, there is no longer a one-to-one relationship between stimulus and muscle response, although such a relationship does exist during the rising phase of the contraction (figure 7*b*). Later, during the maintenance of the tension plateau, there are irregular increases in tension, associated with depolarization, but neither of these events is in any obvious way related to the stimuli (figure 7*c*).

During the first 20 s of repetitive stimulation the amount of tension recovered following a quick release is 90 to 100 % of the tension normally developed at the shorter muscle length (Lowy & Millman 1959*a*; Jewell 1959*b*; Johnson & Twarog 1960). After longer periods of stimulation, less tension is recovered after release, indicating that a maximum

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intensity of 'active-state' is not maintained for long by this method of stimulation (Lowy & Millman 1959*a*). We have also found, in agreement with Fletcher (1937*b*) and Abbott & Lowy (1958*a*), that during repetitive stimulation tension begins to fall 1 to 4 min after the start of such stimulation.

Using Ritchie's (1954*a*) method for measuring the plateau of maximum contractile activity following each shock of repetitive stimulation, we have found that in the *ABRM* tension begins to fall 0·33 s after the last stimulus (average of three experiments) (figure 8). When measured with the same apparatus, the latent period is about 0·04 s; thus, the duration of maximum contractile activity is about 0·29 s. This agrees well with our estimate of fusion frequency in the phasic response, i.e. 4 shocks per second, which would give a duration of maximum contractile activity of 0·25 s.

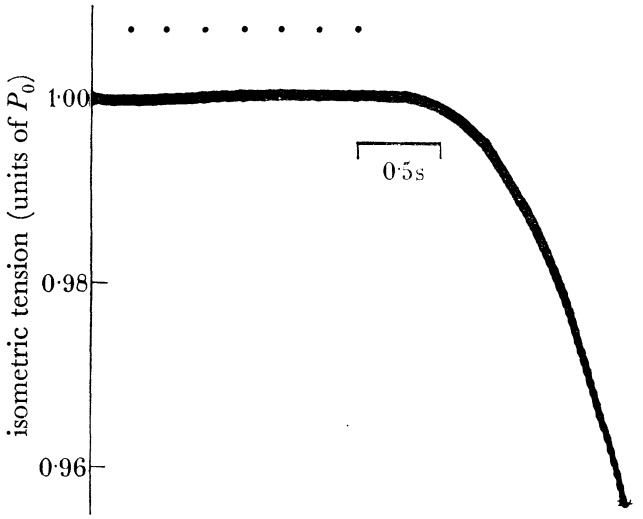

FIGURE 8. Onset of isometric tension decay in the *ABRM* of *Mytilus* after repetitive stimulation at a frequency of about 4 shocks per second. Stimuli recorded above the tension trace. Note that tension starts to fall 0·29 s after the last stimulus. Temperature = 23 °C, length = 2·5 cm, weight = 27 mg.

The effects of changing the extent or speed of release during repetitive stimulation are similar to those observed in a twitch (§3 (*b*)). For releases up to 0·2 l_0 in extent, the amount of tension recovered is independent of the speed of release over the range from 0·01 to 4·0 cm/s. This result agrees with that reported by Jewell (1959*b*), but is contrary to that of Abbott & Lowy (1958*a*), who found that less tension is recovered with slower speeds of release. We have found that the results of Abbott & Lowy can be obtained with muscles in poor condition.

As found by Jewell (1959*b*), the extent of release influences the amount of tension recovered, less tension being recovered for greater extents of release. Typical results from our experiments show recoveries of 95 and 75 % of the tensions normally developed at the shorter lengths for releases of 0·04 and 0·2 l_0 respectively.

(*e*) *The relaxation phase of the phasic response*

In comparison with the twitch response, relaxation after repetitive stimulation is always more rapid. The latter part of the tension decay curve is exponential (figure 9, curve 1), and the values obtained for the time constant range from 1·3 to 6·5 s (19 muscles, average

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value = 3·0 s). This time constant is the same as that obtained for the exponential part of the twitch tension decay curve in a 5 *HT*-treated muscle (§3(*c*); and figure 9, curve 2), suggesting that in these two cases the underlying mechanism of relaxation is the same.

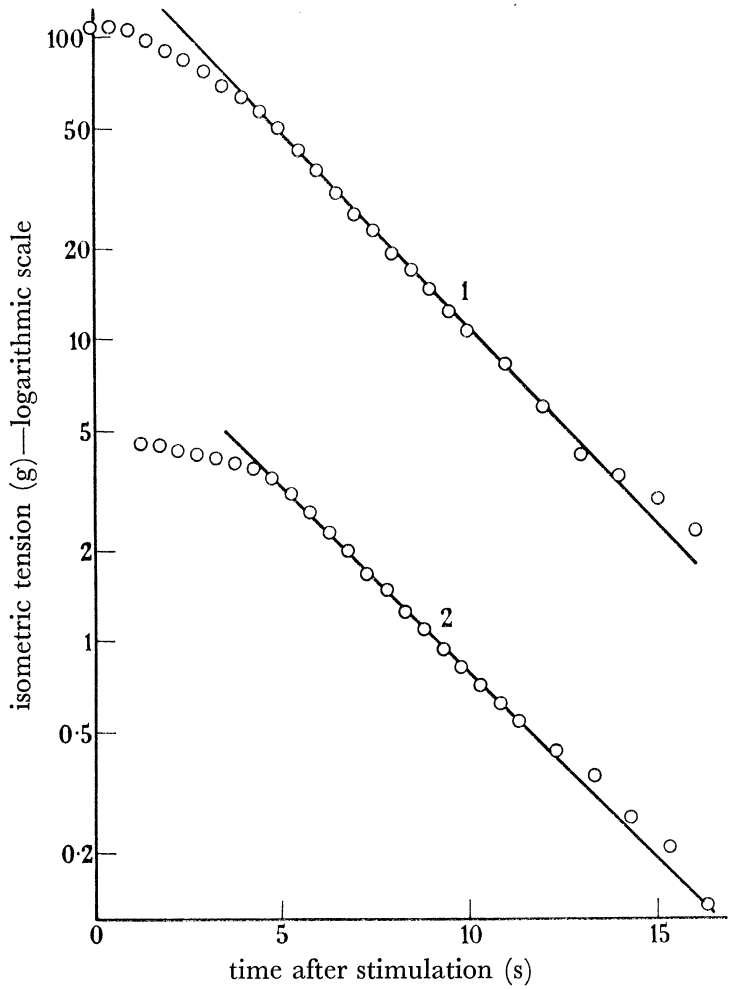

FIGURE 9. Decay of isometric tension in the *ABRM* of *Mytilus* after repetitive stimulation at a frequency of 10 shocks per second (curve 1), and in a twitch in the presence of 10^{-6} g/ml. 5 *HT* (curve 2). Time constant of exponential part of tension decay for repetitive stimulation = 3·4 s, for twitch = 3·5 s. Temperature = 22 °C, length = 2·6 cm, weight = 35 mg.

The time course of tension decay after repetitive stimulation is little affected by 5 *HT*, particularly if muscles are used in which the exponential part of the curve has a time constant less than about 3 s. In muscles with a greater time constant, no change is observed in 10^{-6} g/ml. 5 *HT*, but an increase in the concentration of 5 *HT* from 10^{-6} to 10^{-5} g/ml. may decrease the time constant by a factor of about 2. In such muscles, for the same change in 5 *HT* concentration, a similar change in time constant is also observed in the twitch response. (In comparison, the time constants for tonic and for phasic relaxation differ by a factor of more than 100; see above and §3(*f*).)

For both types of muscle, there is no change in time constant on increasing the concentration of 5 *HT* from 10^{-5} to 10^{-4} g/ml. This suggests that at an external 5 *HT* concentration of 10^{-5} g/ml. the relaxant is present at the reactant sites (see §4(*e*)) in the same

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concentration as during repetitive stimulation (at 4 to 10/s), and that under these conditions the maximum possible rate of relaxation is obtained.

In some instances we have noted that at a concentration of 5 *HT* of 10^{-5} g/ml., the tension developed in response to repetitive stimulation is reduced (e.g. see table 3). It is possible that, under certain conditions, 5 *HT* reduces the amount of contractile activity produced by electrical stimulation (see §3(*c*)).

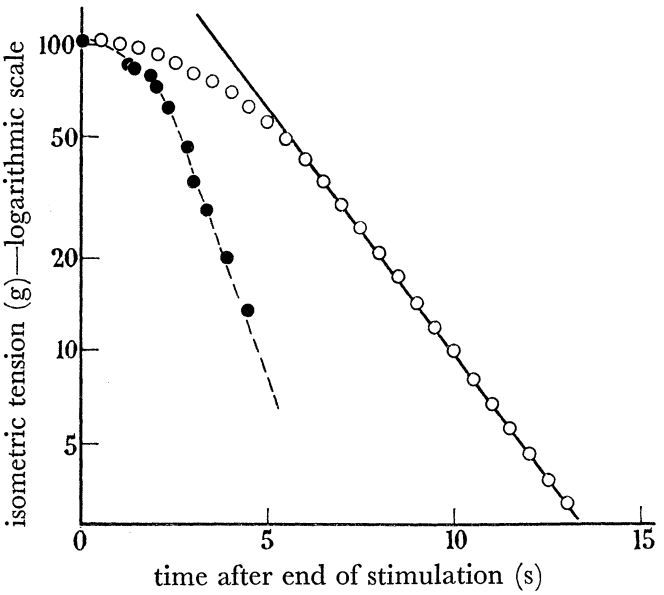

FIGURE 10. Decay of isometric tension (○) in the *ABRM* of *Mytilus* after repetitive stimulation at a frequency of 10 shocks per second. 'Active-state' (●) determined by measuring the tension redeveloped after releases of 0·12 cm at a speed of 1·0 cm/s, given at different times before and after the end of stimulation. Time constant of exponential part of tension decay = 2·7 s. Temperature = 20 °C, length = 2·4 cm, weight = 32 mg.

Using Ritchie's (1954*b*) quick-release method, we have determined the time course of decay of 'active-state' at the end of a phasic response produced by repetitive stimulation in which the maximum intensity of 'active-state' was present, i.e. after less than 20 s of stimulation (figure 10). As might be expected, the time course of decay of 'active-state' is similar to that determined for the twitch response in the presence of 5 *HT* (figure 6). In both these types of response the beginning of the exponential part of the tension decay curve occurs about 5 s after the end of stimulation, and in both responses this coincides with the time when 'active-state' has fallen to a small value (see §3(*b*)).

(*f*) *The tonic response*

Tonic contractions (see figure 1*b*, *c*), produced either by stimulation with d.c. or by *ACh* differ only in detail. In both responses tension rises to a plateau at about the same rate. After stimulation is over, tension decay is usually fairly rapid at first, but becomes much slower before half-peak tension is reached. In these responses too, the latter part of the tension decay curve is exponential. This was shown for the d.c. response by Winton (1937, figure 3), and is here shown for the *ACh* response in figure 11. The values obtained for the time constant of the exponential part of the tension decay curve (in both *ACh*

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and d.c. responses) range from 5 to 100 min, a range similar to that obtained for the twitch response (see §3(*a*)). In view of this we consider the twitch response to be essentially a tonic contraction.

When 5 *HT* (10^{-5} to 10^{-6} g/ml.) is applied to a muscle several minutes after the end of *ACh* stimulation, i.e. long after contractile activity is over (see §3(*g*)), the tension decays rapidly (figure 14*a*). A few seconds after the application of 5 *HT* (provided the muscle is sufficiently thin to allow 5 *HT* to diffuse into all the fibres) the tension decay becomes exponential, with a time constant similar to that for a phasic response, i.e. about 6 s (see §3(*e*)).

In figure 14, relaxation after application of 5 *HT* is slower than that following repetitive stimulation. That this is because of the time taken for the diffusion of 5 *HT*, is evident when muscles of different cross-section are compared, i.e. the thinner the muscle, the faster the

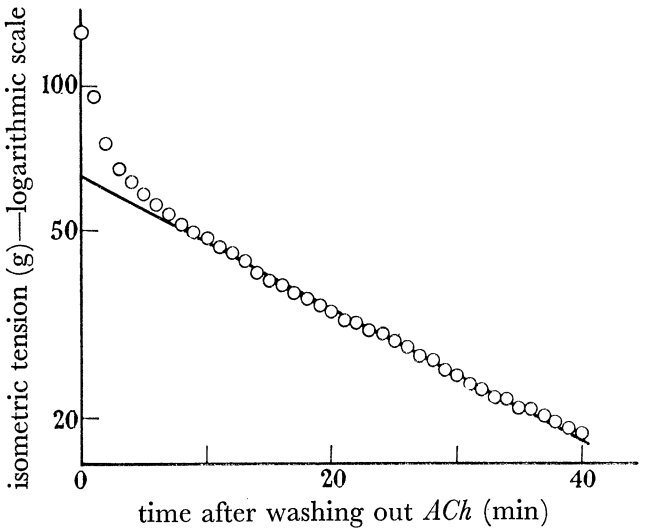

FIGURE 11. Decay of isometric tension in the *ABRM* of *Mytilus*, produced by 10^{-5} g/ml. *ACh* applied for 1 min. Time constant of exponential part of tension decay = 31 min. Temperature = 19 °C, length = 2·7 cm, weight = 41 mg.

relaxation after application of 5 *HT*. Where diffusion is not a limiting factor, as in a twitch in the presence of 5 *HT*, the time constants for the exponential part of tension decay after repetitive stimulation and in the presence of 5 *HT* are the same (see figure 9).

As shown by Jewell (1959*b*), we found that the tension developed in response to the application of *ACh*, using concentrations of 10^{-4} to 10^{-5} g/ml., was always greater than P_0; tensions as great as 14 kg/cm^2 were obtained.

Fletcher (1937*b*) showed that the response of the *ABRM* to d.c. stimulation was localized at the cathode. In our experiments, however, the d.c. response was uniform along the muscle's length, so far as could be detected by visual observation. Nevertheless, with this type of stimulation the amount of tension produced varied considerably, even in different contractions of the same muscle, but was usually within the range from 0·5 to 1·0 P_0. We also found, in agreement with Jewell (1959*b*), that this type of stimulus tends to damage the muscle; it was seldom possible to produce more than 3 or 4 d.c. responses without severely reducing the amount of tension developed in subsequent contractions.

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For these reasons contractions produced by d.c. are not suitable for quantitative comparisons. Therefore, when such comparisons were to be made, *ACh* was used to produce tonic contraction.

(*g*) '*Active-state*' *in the tonic response*

The isometric quick release technique of Ritchie (1954*b*) was used to measure 'active-state' during and after tonic stimulation. As in the case of the phasic contraction, release during stimulation was followed by a recovery of between 80 and 100% of the tension normally developed at the shorter length (Lowy & Millman 1959*a*; Jewell 1959*b*; Johnson & Twarog 1960). This suggests that, as in the phasic response, the muscle attains the maximum intensity of 'active-state' during tonic stimulation.

In a phasic contraction 'active-state' has reached a small value about 5 s after the last shock (§3(*e*)). In a tonic contraction produced by d.c., 'active-state' decays rapidly after the end of stimulation, but not as rapidly as in a phasic response (Lowy & Millman 1959*a*). In most cases, it has dropped to a low value (probably due to damped elastic muscle elements; see §§3(*b*) and 4(*f*)), a minute or so after cessation of stimulation, although tension may not decay to zero until an hour or so later (see also Jewell 1959*a*, *b*; Johnson & Twarog 1960). As in a twitch response (see §3(*b*)), the tension present after contractile activity is over will be called passive tension.

(*h*) *Comparison of phasic and tonic responses*

Table 2 shows the results of a series of experiments on six different muscles in which the peak tension, the shortening speed at zero load, the rate of isometric tension rise, and the compliance of the undamped series elastic component are compared during phasic and tonic stimulation, and in the presence and absence of 5 *HT*.

It is seen that isometric tension is maximal during stimulation with *ACh*, and even in the presence of 5 *HT* this tension is still significantly greater than that developed with repetitive stimulation. The amount of tension produced by *ACh* depends on the concentration *ACh* used. Cambridge, Holgate & Sharp (1959) showed that at a concentration of 5×10^{-5} g/ml. the tension–concentration curve is still rising. We have found that using 10^{-3} g/ml. *ACh* a further increase in tension is obtained, which is about 10% above that obtained when the concentration is 10^{-4} g/ml. But after treatment with 10^{-3} g/ml. *ACh* the muscle shows signs of irreversible damage (e.g. tension does not decay completely, even on application of 5 *HT*). For this reason we have not used *ACh* in concentrations above 10^{-4} g/ml., at which concentration the muscle shows no signs of damage.

From the above considerations it would appear that the observed differences between the isometric tension developed with repetitive stimulation and that developed with *ACh* is largely a matter of the concentration of *ACh* used. But there are other factors involved. On the assumption that tension in the *ABRM* is produced by linkages in a sliding filament mechanism (discussed in detail in §4(*a*)), the maximum tension maintained will depend on the rate of formation of linkages. But as has been pointed out to us by A. F. Huxley (personal communication) the rate at which linkages break will have a second order effect, in that the greater the linkage breaking rate, the less the tension maintained. Thus, in the presence of 5 *HT* when the linkage breaking rate is assumed to be high, it is expected that less tension would be maintained than when the linkage breaking rate is low, as is the case

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in a tonic response. We have, in fact, observed that in the presence of 10^{-5} g/ml. 5 *HT* the tension produced by 10^{-4} g/ml. *ACh* is reduced by 7 % P_0. That this effect is not caused by other factors (e.g. a depression of muscle activity by 5 *HT*) is indicated by the finding that during maximum contractile activity the amount of tension produced by repetitive stimulation in these particular muscles was not significantly affected by 5 *HT* (table 2).

Table 2 also shows that there is no significant difference in the shortening speed at zero load during tonic and phasic stimulation.

TABLE 2. COMPARISON OF PHASIC AND TONIC CONTRACTIONS OF THE *ABRM* OF *MYTILUS* DURING MAXIMUM CONTRACTILE ACTIVITY

Six different muscles; temperature = 20 °C; average l_0 = 2·5 cm; average weight = 27 mg.

	peak isometric tension	shortening speed at zero load	maximum rate of tension rise (initial)	maximum rate of tension rise (after a release of 0·12 l_0)	total compliance of the series elastic component
(average values during repetitive stimulation) (10 shocks per second)	P_0 = 8·6 kg/cm²	V_0 = 0·25 l/s	0·52 P_0/s	0·45 P_0/s	4·3 % l_0
		relative values			
repetitive stimulation (10 shocks per second)	1·00	1·00	1·00	1·00	1·00
repetitive stimulation (10 shocks per second, in the presence of 10^{-5} g/ml. 5 *HT*)	0·99 ±0·002*	1·02 ±0·04	0·77 ±0·04	0·92 ±0·04	0·96 ±0·04
ACh stimulation (10^{-4} g/ml.)	1·22 ±0·02	0·99 ±0·03	0·88 ±0·09	1·00 ±0·04	1·14 ±0·05
ACh stimulation (10^{-4} g/ml., in the presence of 10^{-5} g/ml. 5 *HT*)	1·15 ±0·03	1·09 ±0·05	0·71 ±0·04	0·97 ±0·07	1·10 ±0·02

* Standard error of the mean of six observations.

The finding that the initial rate of rise of tension is slower during tonic stimulation with *ACh* than during repetitive stimulation is probably due to the limiting effect of the rate of diffusion of *ACh* into the muscle. In tonic responses produced by d.c., the initial rate of rise of tension is almost the same as in a phasic response. During *ACh* stimulation, the rate of rise of tension after a release is the same as during repetitive stimulation. It has been shown (Lowy & Millman 1959*a*; Jewell 1959*b*) that the rate of rise of tension in response to repetitive stimulation as well is higher after the release than initially, an observation which appears to be contradicted by the results in table 2. This is because the extent of release in the experiments of table 2 was considerably greater than that required to drop tension to zero. Under such conditions less tension is redeveloped (see §3(*d*)), and it is thus not surprising that tension rises more slowly.

The greatest effect of 5 *HT* (not shown in table 2) is on the decay rate of the tonic response, which is increased by a factor of about 100 (see also §3(*f*)). The presence of 5 *HT* has no significant effect on any of the parameters shown in table 2, with the exception of the initial rate of rise of tension, which is reduced. This could be explained on the

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assumption that 5 *HT* has a depressing effect on the *development of contractile activity* in the muscle, an idea also implied by the suggestion that in the presence of 5 *HT* less contractile activity is produced in response to a single stimulus (see §3(*c*)).

The last column of table 2 compares the compliance of the undamped series elastic component under the various conditions. The total compliance was measured by determining the change in muscle length (extent of release) required to drop tension to zero during maximum contractile activity while the muscle was maintaining peak tension.

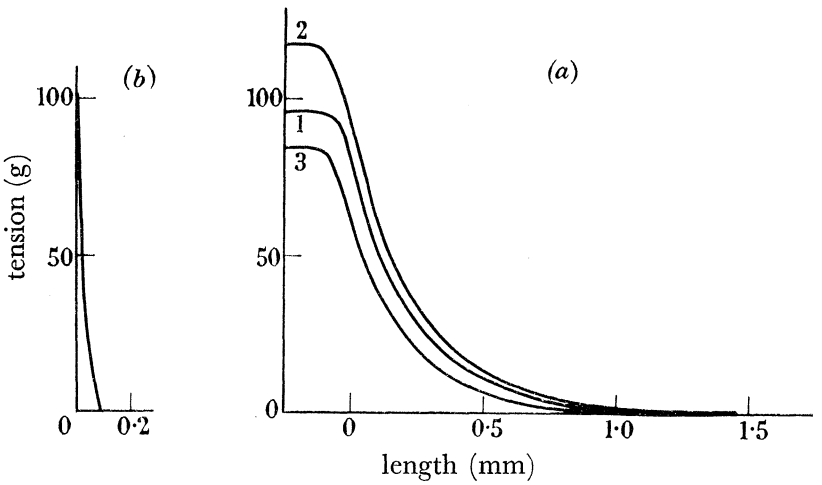

FIGURE 12. The series elastic effect in the *ABRM* of *Mytilus*. (*a*) Tracings of records obtained by releasing the muscle from 2·20 to 2·05 cm at a constant speed of 4·4 cm/s: (1) during repetitive stimulation at a frequency of 10 shocks per second, (2) during stimulation with 10^{-5} g/ml. *ACh*, (3) after stimulation with 10^{-5} g/ml. *ACh*, i.e. 1 min after washing the muscle with fresh bathing solution. Curves are not corrected for the compliance of the apparatus and the connexions to the muscle, or the active shortening during the release (maximum shortening speed of the muscle = 0·6 cm/s). The stress–strain curve for the apparatus (including the connexions to the muscle) is shown in (*b*). (The slope of the release curves is less at the start of the release because the Levin–Wyman ergometer does not reach constant speed immediately.) Temperature = 21 °C, l_0 = 2·4 cm, weight = 19·5 mg.

The stress–strain curve of the undamped series elastic component was determined by the controlled-release method of Hill (1950) using very rigid apparatus (see Methods). Typical curves obtained during *ACh* and repetitive stimulation, and also after *ACh* stimulation are shown in figure 12*a*. For comparison, the stress–strain curve of the apparatus (including connexions to the muscle) is shown in figure 12*b*. These curves are similar to those obtained by Jewell (1959*b*, 1960) during repetitive stimulation and after *ACh* stimulation. From table 2 it appears that the total compliance of the series elastic component is greater during tonic than during phasic stimulation, but this is because the series elastic elements are stretched more by the greater tension produced by *ACh* stimulation. That this is so can be clearly seen in figure 12.

(*i*) *The relation between isometric tension and muscle length*

The isometric tension–length curve for the phasic contraction has been described previously (Abbott & Lowy 1958*b*). We have extended this work, and in addition studied the amount of tension developed at different muscle lengths in the tonic response. Our

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experiments show that during stimulation in either type of response, tension is actively developed over a range of lengths which may extend from 0·2 l_0 to 1·5 l_0 (figure 13). *We define l_0 as the shortest length of the muscle at which a definite resting tension can be detected.* This gives a consistent value for l_0 (with respect to the tension–length curve), which is slightly greater than that used by Abbott & Lowy (1958*b*) and Jewell (1959*b*). However, the difference is not serious because near l_0 there is little variation of active isometric tension with length (cf. figure 13).

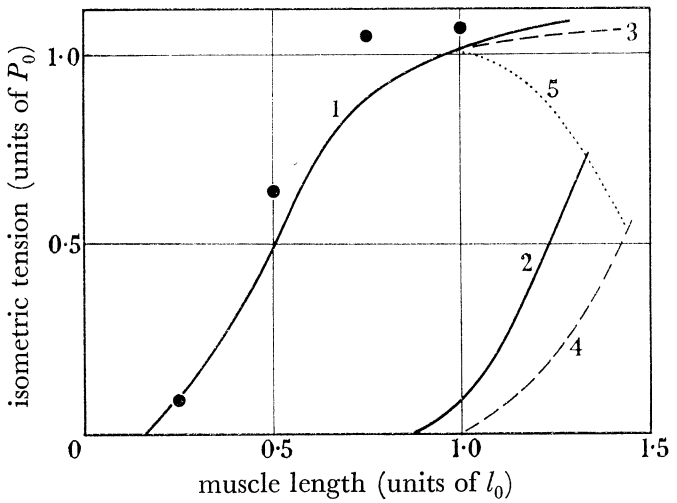

FIGURE 13. Isometric tension–length relationship of the *ABRM* of *Mytilus* at 20 °C. Twelve muscles were used for these experiments. Each curve was obtained by averaging the results from seven to ten muscles. (Because of the large number of points required, it was not always possible to obtain all curves for each muscle.) The solid curves represent experiments done in sea water: curve (1) shows the total tension during repetitive stimulation at a frequency of 10 shocks per second, and curve (2) the apparent resting tension (for explanation see §3(*j*)). The dashed curves represent experiments done in sea water with 5 *HT* (10^{-5} to 10^{-6} g/ml.): curve (3) shows the total tension during repetitive stimulation, and curve (4) the resting tension. Curve (5) was obtained by subtracting curve (4) from curve (3), and therefore represents the active tension developed in response to repetitive stimulation. ● represent the tension developed with 10^{-5} g/ml. *ACh* in sea water. For clarity, the points for repetitive stimulation and *ACh* stimulation in the presence of 5 *HT* below l_0 are not shown; they lie slightly below the points determined in sea water.

(*j*) *The presence of passive tension above l_0*

When the unstimulated muscle is extended above l_0, a tension usually appears which decays slowly, and reaches half its peak value in about 30 min. We propose to call this tension *apparent resting tension* to distinguish it from true resting tension, which is probably due to inert tissue in parallel with the contractile apparatus.

We have found that the amount of apparent resting tension present at any particular muscle length above l_0 varies, in that it depends on the previous history of the muscle. The part of apparent resting tension that is due to true resting tension can be determined by treatment of the muscle with 5 *HT* (10^{-5} to 10^{-6} g/ml.). This rapidly reduces the tension to a lower level (figure 14*b*). The tension now remains at the lower level, and only drops extremely slowly over a period of days. It is in no way affected either by stimulating the

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muscle, or by abolishing excitability with $MgCl_2$. Thus, this tension appears to be similar to resting tension in the frog's sartorius muscle.

What is the nature of that part of the apparent resting tension which can be abolished by 5 *HT*? It is similar to the tension produced by stretch of the smooth adductor of *Pecten*. In the *Pecten* muscle, Bozler showed that tension produced by stretch and tension produced by electrical stimulation are the same (Bozler 1930—discussed in §4(*l*)). We have repeated Bozler's observations using the *ABRM*, and made some additional ones.

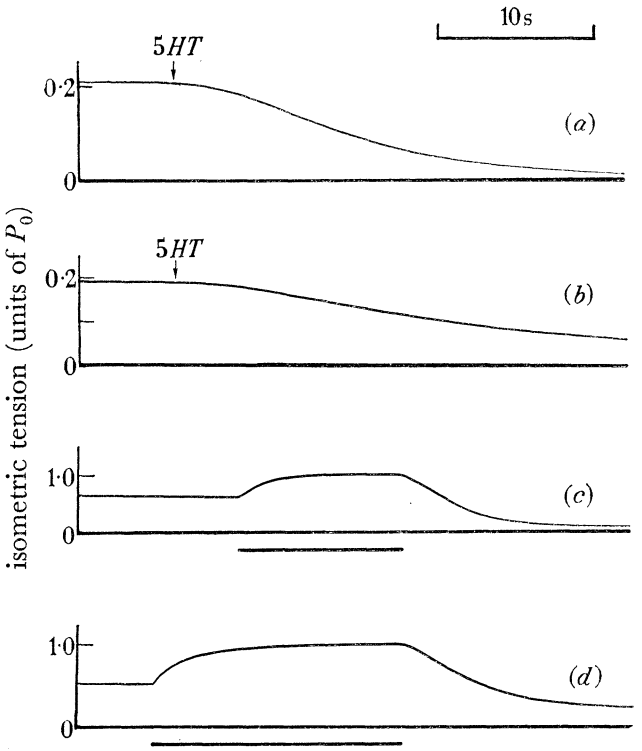

FIGURE 14. The effects of 5 *HT* and repetitive stimulation on isometric passive tension in the *ABRM* of *Mytilus* at 20 °C. (Records from four different muscles.) (*a*) Tension developed with 10^{-5} g/ml. *ACh*, relaxed by 10^{-5} g/ml. 5 *HT* applied at the arrow; (*b*) tension produced by extension of the resting muscle to 1·07 l_0, partly relaxed by 10^{-5} g/ml. 5 *HT* applied at the arrow; (*c*) tension developed with 10^{-5} g/ml. *ACh*, relaxed by repetitive stimulation at a frequency of 10 shocks per second; (*d*) tension produced by extension of the resting muscle to 1·10 l_0, partly relaxed by repetitive stimulation at a frequency of 10 shocks per second. The period of repetitive stimulation is indicated by a solid line below the traces in (*c*) and (*d*).

The following results, obtained with the *ABRM*, indicate (as originally suggested by Bozler) that part of the apparent resting tension is identical with what we have called passive tension:

(1) both tensions decay to half value in about 20 min (cf. Bozler 1930);

(2) both tensions are reduced by repetitive shocks (figure 14*c*, *d*; cf. Bozler 1930);

(3) both tensions are affected similarly by 5 *HT*, which accelerates their decay so that half-peak value is reached in 5 to 10 s (figure 14*a*, *b*);

(4) neither tension can be produced in a muscle treated with isotonic $MgCl_2$ (0·36 M).

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As regards point (2), we have found in some cases that apparent resting tension is not as effectively reduced by repetitive shocks as is passive tension. In such instances it is probable that the apparent resting tension would be reduced further were it not for the presence of spontaneous contractile activity which usually follows repetitive stimulation of a muscle at lengths above l_0. The amount of this activity varies; often it is so pronounced that it is quite evident in the tension records (figure 15*a*, *b*). Often, contractions occurring regularly once every 5 to 20 s have been observed, particularly in the presence of 5 *HT* (figure 15*c*, *d*).

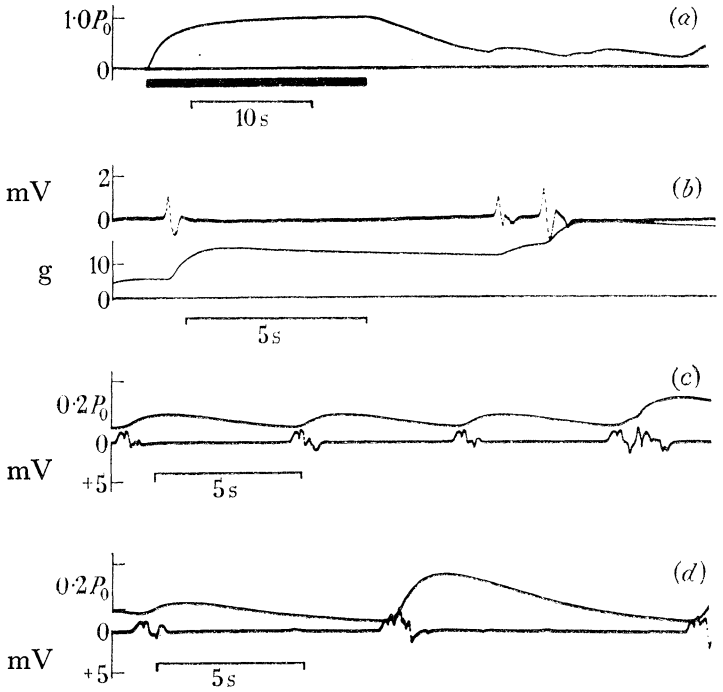

FIGURE 15. Spontaneous activity in the *ABRM* of *Mytilus*. (*a*) Response to repetitive stimulation at l_0 (10 shocks per second), also showing spontaneous contractions following the stimulation. The period of stimulation is indicated by the heavy line below the tension trace. Temperature = 21 °C, l_0 = 3·4 cm, weight = 32 mg, P_0 = 75 g. (*b*) Spontaneous contractions after repetitive stimulation (10 shocks per second) at 1·15 l_0. Electrical activity on upper trace, isometric tension on lower trace. Temperature = 24 °C, l_0 = 3·1 cm, weight = 32 mg, P_0 = 78 g. (*c*) and (*d*) same muscle as (*b*); spontaneous contractions in the presence of 10^{-5} g/ml. 5 *HT*, recorded several minutes after repetitive stimulation (10 shocks per second) at 1·25 l_0. Isometric tension on upper traces, electrical activity on lower traces.

With reference to point (4), we found that after treatment with isotonic $MgCl_2$ the *ABRM* cannot be excited by the usual methods of stimulation (repetitive shocks, d.c., *ACh*), and that the resistance of the unstimulated muscle to a rapid stretch above l_0 is greatly reduced (see §3(*m*)). If $MgCl_2$ is applied for only a few minutes, its effects are almost completely reversible: after longer application, the effects are only partly reversible.

(*k*) *Active tension above* l_0

The finding that apparent resting tension is made up of the sum of passive tension and true resting tension leads to the possibility of determining the amounts of these tensions and of active tension which the muscle can develop at any length. Figure 13 shows this

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and also reveals that at any given length above l_0 the total tension is always about the same, although there may be large variations in the amount of initial (passive) tension present when the muscle is stimulated. This point is specifically illustrated in the experiment shown in the records of figure 16. For this experiment, the more rigid apparatus (with a compliance as shown in figure 12*b*) was used. First a muscle at 1·35 l_0, holding an apparent resting tension of 19·4 g, was stimulated with repetitive shocks. This produced an active tension of 8·8 g, making a total tension of 28·2 g. After repetitive stimulation the

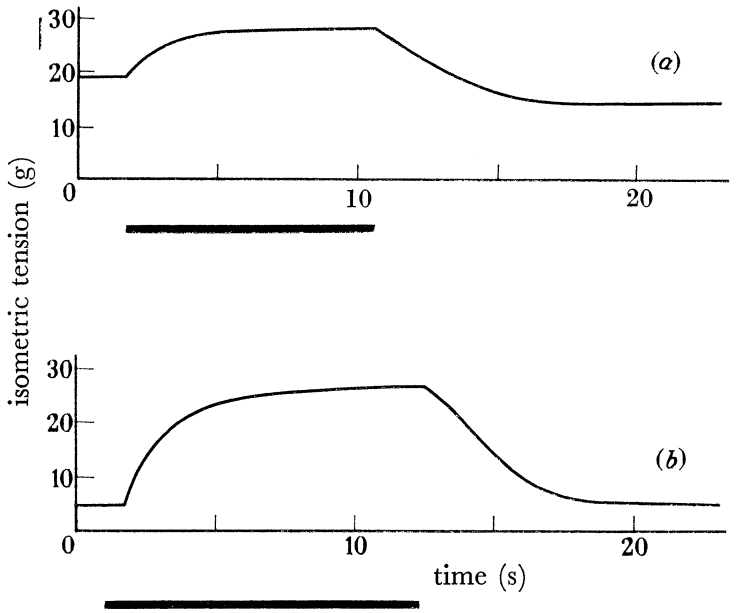

FIGURE 16. Isometric active tension developed in the *ABRM* of *Mytilus* when the muscle is holding different amounts of apparent resting tension (see §3(*j*)) at the same muscle length (3·1 cm). Tracings of original records. The period of stimulation is shown by a solid line below each trace. (*a*) Muscle stimulated with repetitive shocks at a frequency of 10 shocks per second while holding 19·4 g of apparent resting tension. (*b*) Experiment similar to (*a*), but in the presence of 10^{-6} g/ml. 5 *HT*, by means of which the passive tension has been abolished. Note that in (*a*), repetitive stimulation relaxes only part of the passive tension held before stimulation, and that the total tension is very nearly the same in (*a*) and (*b*), i.e. 28·2 and 26·9 g respectively. Temperature = 20 °C, l_0 = 2·3 cm, weight = 12·8 mg.

tension decayed to a value of 14·7 g. The muscle was then treated with 5 *HT*, which abolished the passive tension, leaving a true resting tension of 4·1 g. Repetitive stimulation now produced an active tension of 22·8 g, about 2·5 times more than previously. The total tension was now 26·9 g., 5 % less than the total tension produced in the untreated muscle.

(*l*) *Definitions of types of tension*

At this stage it may be useful to define the various types of tension observed in the *ABRM*:

(1) *Active tension*, due to stimulation; characterized by the presence in the muscle of contractile activity, i.e. the ability to shorten and develop tension actively.

(2) *Passive tension*, due either to previous contractile activity or to extension of the unstimulated muscle; characterized by the absence of contractile activity, a very low decay rate, and the fact that it can be abolished by 5 *HT*.

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(3) *Resting tension*, present at muscle lengths above l_0, and presumably due to inert elastic material in parallel with the contractile apparatus; characterized by a negligible decay during the normal course of an experiment, and by the fact that it cannot be abolished by 5 *HT* or $MgCl_2$.

(4) *Stretch tension*, produced by rapidly stretching the stimulated or unstimulated muscle. The phenomena involved are characterized in the two following sections.

(*m*) *Responses to stretch*

When the contracted *ABRM* (i.e. during or after stimulation, while the muscle is holding tension) is stretched at constant speed, at lengths above or below l_0, the tension increases during the stretch, and then decays after the stretch to a value greater than that

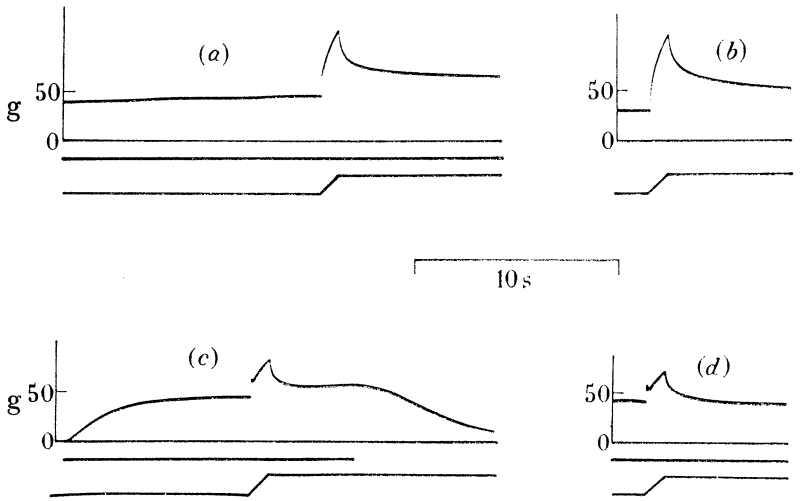

FIGURE 17. Effects of stretching the contracted *ABRM* of *Mytilus* at constant speed. Tension shown on upper traces, length on lower traces. Stimulation indicated by the solid line between the tension and length traces. Muscle stretched from 2·0 to 2·2 cm at a speed of 0·2 cm/s: (*a*) during tonic stimulation with 10^{-5} g/ml. *ACh*; (*b*) 1 min after tonic stimulation with 10^{-5} g/ml. *ACh*; (*c*) during repetitive stimulation at a frequency of 5 shocks per second, and (*d*) during stimulation with 10^{-5} g/ml. *ACh* in the presence of 10^{-5} g/ml. 5 *HT*. Temperature = 19 °C, l_0 = 2·4 cm, weight = 34 mg.

which would be present at the same time interval after the stimulus in a similar contraction (without stretch) at the longer length. We have observed the increment of tension which remains after the stretch in nearly all experiments in which a contracted muscle was stretched. The form of the tension–time curves during the stretch differs in phasic and tonic contractions as shown in figure 17.

During stretch of the resting muscle, as in the case of the contracted muscle, tension increases during the stretch and decays afterwards. If the muscle is stretched at lengths below l_0, tension will eventually decay to zero, although the latter part of this decay may be very slow (see §3 (*n*)). The stretch tension obtained is very variable, and always less than that obtained during contraction, although values up to 0·5 P_0 have been observed. Tension produced by stretch of the resting muscle is greatly reduced in the presence of 10^{-5} to 10^{-6} g/ml. 5 *HT*, or of isotonic $MgCl_2$.

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In table 3 the magnitude of the stretch response at lengths below l_0 is compared for various conditions: at rest, during phasic stimulation, during and after tonic stimulation produced by either d.c. or *ACh*, and during *ACh* stimulation in the presence of 5*HT*. The table gives the results from three series of experiments (*a*, *b* and *c*), and in each series the figures represent the average of several results from two muscles. The tension present at the start of the stretch (*initial tension*) is compared with the tension added by the stretch (*stretch tension*). It is seen that stretch tension is greater during tonic than during phasic

TABLE 3. TENSION DEVELOPED DURING STRETCH OF THE *ABRM* OF *MYTILUS* UNDER VARIOUS CONDITIONS BELOW l_0

Three series of experiments (*a*, *b* and *c*), each showing the averages of several results from two muscles. Temperature = 20 °C.

	amount of stretch (% l_0)	speed of stretch (% l_0/s)	initial tension (% P_0)	stretch tension (% P_0)
	a			
at rest	6·0	7·3	0	22
during repetitive stimulation (10/s)	6·0	7·3	100	46
during d.c. stimulation	6·0	7·3	76	68
after d.c. stimulation	6·0	7·3	48	64
	b			
at rest	9·6	9·6	0	4·4
during repetitive stimulation (10/s)	9·6	9·6	100	88
during *ACh* stimulation (10^{-5} g/ml.)	9·6	9·6	116	135
after *ACh* stimulation (10^{-5} g/ml.)	9·6	9·6	86	135
during repetitive stimulation (in the presence of 10^{-5} g/ml. 5 *HT*)	9·6	9·6	68	64
during *ACh* stimulation (in the presence of 10^{-5} g/ml. 5 *HT*)	9·6	9·6	92	58
	c			
at rest	0·8	0·8	0	2
during repetitive stimulation (10/s)	0·8	0·8	100	13
during *ACh* stimulation (10^{-5} g/ml.)	0·8	0·8	110	20
after *ACh* stimulation (10^{-5} g/ml.)	0·8	0·8	68	23
during repetitive stimulation (in the presence of 10^{-5} g/ml. 5 *HT*)	0·8	0·8	78	8
during *ACh* stimulation (in the presence of 10^{-5} g/ml. 5 *HT*)	0·8	0·8	90	10

stimulation, but during tonic stimulation in the presence of 5 *HT*, the amount of stretch tension is similar to that during phasic stimulation. After tonic stimulation, the stretch tension may be as great or greater than during the tonic stimulation, even though the initial tension is considerably less in the former case. The sum of stretch and initial tension, however, is always greater during than after tonic stimulation.

For stretches of about 0·1 l_0, the stretch tension increases with speed of stretch by a factor of 3 to 5 over the range from 0·01 to 1·0 l_0/s. This has been found for muscles at rest, and during both phasic and tonic (d.c.) stimulation.

Johnson & Twarog (1960, p. 952) have used the term *stiffness* in describing the results of stretch experiments similar to our own. They consider the *ABRM* to be stiffer 'under one set of conditions than it is under another (set) if, when stretched by the same amount in both cases, it develops and maintains a greater tension in the first case'. In our

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terminology, a greater stiffness would be indicated by a greater value for the stretch tension and for the increment of tension which remains after the stretch. We have found that this increment and the stretch tension always vary similarly under the different conditions studied. It is clear, therefore, that in our experiments, stretch tension can be taken as a measure of stiffness in the sense used by Johnson & Twarog.

(*n*) *Stress relaxation*

The decay of tension following stretch of a resting muscle (stress relaxation) has been analyzed previously (Bozler 1930, 1931; Abbott & Lowy 1957, 1958*b*). We have shown that in the *ABRM* at lengths below l_0, the stress relaxation curve can be resolved into three exponential components (Lowy & Millman 1959*b*). The decay rate of the slowest component was found to be similar to the rate of decay of isometric tension in a tonic response:

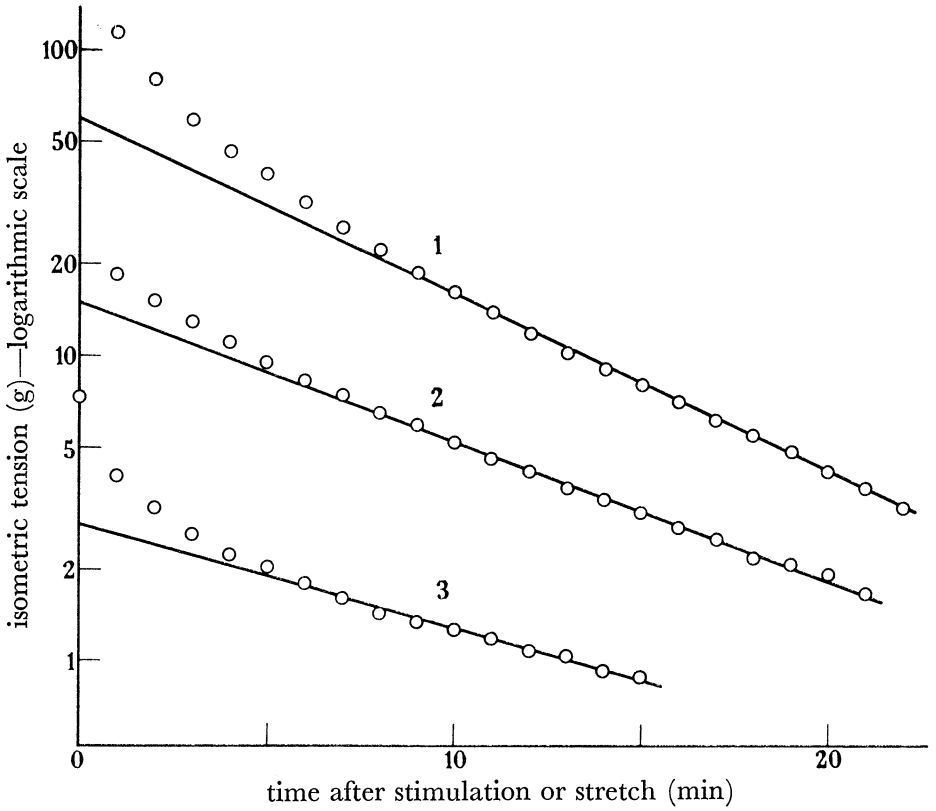

FIGURE 18. Decay of tonic tension in the *ABRM* of *Mytilus*. Curve (1) shows the decay of isometric tension produced by applying 10^{-4} g/ml. *ACh* to the muscle at 1·6 cm; curve (2) shows stress relaxation (see §3(*n*)) after a constant speed stretch from 1·3 to 1·6 cm at a speed of 0·2 cm/s; curve (3) shows the decay of isometric tension following a single stimulus at 1·6 cm. Time constants of the exponential part of tension decay for curve (1) = 7·5 min, for curve (2) = 9·5 min, for curve (3) = 12·7 min. Temperature = 21 °C, l_0 = 1·8 cm, weight = 31 mg.

this similarity can be seen in figure 18 where stress relaxation, twitch tension decay, and *ACh* tension decay are plotted on a semi-logarithmic scale. The rate of tension decay is almost the same in the three cases 1 min or so after the stretch or stimulus. In the twitch and *ACh* responses it has been shown that the tension present at this time is passive tension (§§3(*b*) and 3(*g*)). Because of the similar decay rates, it seems reasonable to suggest that

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1 min or so after stretch of the resting muscle, the tension present is also passive tension. Supporting evidence for this idea comes from the finding that if the muscle is stretched under conditions which are known to reduce or abolish passive tension, i.e. less than 2 min after phasic stimulation or in the presence of 5 *HT*, stress relaxation shows little or none of the third decay component. This is similar to Bozler's (1930) finding for the smooth adductor muscle of *Pecten*, that repetitive stimulation increases the decay rate of stress relaxation.

We have already shown that stretch of the resting muscle can produce passive tension at lengths above l_0 (§3(*g*)). From the experiments described here we conclude that this is also true at lengths below l_0, i.e. where true resting tension is not involved.

4. Discussion

(*a*) *The linkage hypothesis of tonic contraction*

Recent work on the structure of the *ABRM* and of other lamellibranch muscles (see Hanson & Lowy 1960; Lowy & Hanson 1962) suggests that these muscles contract by a sliding filament mechanism essentially similar to that postulated for vertebrate striated muscle (see Huxley & Hanson 1960). Accordingly we have interpreted the results of our experiments in terms of a sliding filament model, though we are aware that this is not the only possible explanation.

To account for phasic and tonic contractions in the *ABRM*, we previously proposed (Lowy & Millman 1959*b*): (1) that the tension developed during the active state is due to the formation of linkages in a sliding filament actin-myosin system, and that linkages are also made which involve a second system, characterized by the presence of filaments with paramyosin structure; (2) that these latter linkages (tonic linkages) break at a very low rate and are responsible for the phenomenon of passive tension; (3) that activation of 'inhibitory' nerves increases the rate at which the tonic linkages break.

This hypothesis is not very different from that put forward by Johnson *et al.* (1959), Johnson & Twarog (1960), and Rüegg (1959, 1961*a*) who postulate the existence of an actomyosin system acting in parallel with a functionally independent paramyosin system. On such an 'independent catch hypothesis' the process of shortening and/or generation of active tension involves only the actomyosin system; the paramyosin system merely maintains tension and does so by a change in its viscoelastic properties.

However, other results (Lowy & Millman 1959*c*) suggest—instead of our previous 'two-linkage' hypothesis—a simpler explanation, which assumes linkages of one type only, but with a breaking rate which is governed during isometric relaxation by the concentration of relaxant (very likely 5 *HT*) present. During active shortening, however, linkages must detach at a constant and much higher rate, otherwise unbroken linkages would soon arrest motion. The need for a second linkage breaking rate has been shown by A. F. Huxley (1957) in his hypothesis for contraction in vertebrate striated muscle.

The linkages in the molluscan muscle would be similar to actin-myosin linkages, assuming that the thick filaments in the *ABRM* are homologous with the myosin-containing filaments in vertebrate striated muscle. The tension developed and maintained by the muscle is due to the stress exerted by the series elastic elements, particularly the

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undamped series elastic component, on linkages formed during activity. This hypothesis is referred to as the linkage hypothesis. The experimental evidence on which it is based is summarized in the following two sections.

(*b*) *Evidence for the linkage hypothesis from experiments during maximum intensity of 'active-state'*

Before discussing these experiments it is necessary to point out that there is good evidence that the system which is responsible for passive tension is in action during tonic stimulation. As shown in §3(*m*), the muscle's stiffness is greater during tonic than during phasic stimulation, whereas it is similar during and after tonic stimulation. The greater stretch tension during tonic stimulation can be explained on the linkage hypothesis as follows. Since during tonic stimulation linkages break at a lower rate than during phasic stimulation, it is reasonable to suppose that these linkages will also be broken more slowly while the muscle is being stretched during tonic stimulation. This will mean that in comparison with a stretch during phasic stimulation, each linkage will be holding somewhat more tension, and as well there may be more linkages in existence at any particular time (since as explained below, the rate of formation of linkages is the same during tonic and phasic stimulation).

We have found that there is no difference in either the shortening speed at zero load or the maximum rate of tension rise after a release during tonic and phasic stimulation (see table 2). The *amount* of shortening is also much the same during tonic and phasic stimulation (Jewell 1959*b*). These findings are explicable on the linkage hypothesis, though at first sight one might expect the shortening speed at zero load to be less during tonic than during phasic stimulation, on the assumption that this parameter depends on the linkage breaking rate. But, for example, on the particular formulation of the sliding filament hypothesis put forward by A. F. Huxley (1957) (applied to vertebrate striated muscle), it was necessary to postulate two different linkage breaking constants during activity. These operate one on either side of the equilibrium position of each active site. One constant governs the breaking of linkages when muscle length is not changing (isometric contraction) or is increasing. The second constant is necessary so that linkages carried past the equilibrium position during shortening can be broken (at a higher rate); otherwise they would persist indefinitely and motion would cease. Assuming that a similar sliding filament hypothesis can be applied to the *ABRM*, there is no reason to suppose that the second linkage breaking constant (which operates during shortening) would differ during tonic and phasic stimulation. Therefore, the shortening speed at zero load could be the same during either type of response. Other things being equal (e.g. the series elastic component, see §4(*c*)), it follows that the maximum rate of tension rise would be the same too.

On the independent catch hypothesis it would be expected that when the tonic system is in action the muscle is rigid, and therefore unable to shorten. The observation that shortening speed at zero load is identical during tonic and phasic stimulation shows, however, that the tonic system must behave like a ratchet. In terms of the independent catch hypothesis this is possible on either of the following two assumptions:

(i) that the paramyosin system can shorten actively, or

(ii) that the paramyosin system is plasticized when the muscle is allowed to shorten during tonic stimulation.

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If (i) is accepted, the paramyosin system behaves like a second contractile system. But Johnson *et al.* (1959), using the glycerol-extracted *ABRM*, have shown that when the paramyosin system is supposed to be in action such preparations can shorten by only 20 % of the amount possible when the actomyosin system alone is in action.

Assumption (ii) is feasible only if it is assumed that the paramyosin system comes back into action whilst tension is being redeveloped by the actomyosin system. This additional assumption is necessary because we have found that when a tonically stimulated muscle is released (e.g. by 0·1 l_0), the tension redeveloped is tonic tension, i.e. it decays at the low tonic rate when stimulation is stopped. The assumption put forward in (ii) also seems unlikely in view of Jewell's (1959*b*) finding that an *ABRM* holding passive tension can be released and stretched as many as 70 times with almost no drop in the tension maintained at the longer length.

The case for the independent catch hypothesis would be considerably strengthened if it could be shown that the tonic system only comes into action after stimulation has stopped. This might appear to be the implication of Winton's (1935, 1937) experiments with the *ABRM*, though Winton himself concluded (1937, p. 496) that an increased 'viscosity' (as discussed below) is already present *during* tonic stimulation. Winton studied the 'viscosity' and 'compliance' of the muscle by submitting it to sinusoidal tension changes at different frequencies during and after phasic (a.c.) and tonic (d.c.) stimulation. He found that the variation in amplitude of the observed changes in muscle length with frequency is greater during than after stimulation. By comparing the amplitude of length changes at two different frequencies, Winton obtained a measure of the 'viscosity'. He found that 'viscosity' is greatest after tonic stimulation, lowest during phasic stimulation, and of an intermediate value during tonic and after phasic stimulation. Winton's results indicate that the muscle's 'viscosity' decreases during stimulation. Pryor (1950) reached much the same conclusion from similar experiments with the *ABRM*, and also with the frog's gastrocnemius muscle.

Winton's results might appear to disagree with the results of our stretch experiments which show that the muscle's stiffness is greater during stimulation than at rest (see table 3). But an increase or decrease of 'viscosity' in the *ABRM*, as measured by Winton and Pryor, cannot be taken as a direct indication of the presence or absence of the tonic system. In particular, there is the fact that the amplitude of the length changes during stimulation will be affected not only by the resistance to extension but also by the muscle's ability for active shortening. At low frequencies of oscillation the muscle will be able to shorten appreciably during the shortening phase of the oscillatory cycle, thus bringing it into a range of the isometric tension–length curve where less active tension is developed. When the muscle is now stretched during the lengthening phase of the cycle, it will offer less resistance than at the longer length (during the first part of the lengthening phase of the cycle). At higher frequencies of oscillation, the muscle will not be able to shorten so much during a single shortening phase. In addition, because of the resulting greater initial muscle length and the shorter period of time for lengthening, the muscle will not be stretched as much. Thus the amplitude of length change in the *active* muscle will be greater at low than at high frequencies of forced oscillation. This explanation is supported by Winton's (1937) figure 7 which shows that at the low frequency the muscle reaches much shorter lengths than at the high frequency.

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If our explanation of Winton's results is correct, one would expect the frequency dependence of the amplitude of muscle length change during oscillation to bear a definite relationship to the shortening speed of the muscle. That this is so is indicated by Winton's (1937) figure 8 which shows that although the amplitude of length change is greater during than after a.c. stimulation at low frequencies of oscillation, at high frequencies the reverse is true. The frequency at which the amplitudes during and after stimulation are the same is about 10 c/min. During the shortening half of the cycle at this frequency the *ABRM* is allowed to shorten at an average speed which approximates its maximum shortening speed.

That the behaviour of the *ABRM* as described in Winton's experiments is not unique is shown by the fact that Pryor (1950) obtained similar results with the frog's gastrocnemius muscle. Pryor found that the amplitude of the length changes was greater during than after stimulation for all frequencies which he was able to produce with his apparatus, i.e. up to 4 c/s. But Pryor's records indicate that these amplitudes would have become equal at a frequency of oscillation about ten times that of the corresponding frequency found for the *ABRM*. This is the result one would expect, considering the faster shortening speed of the frog's muscle.

Hill (1953) has summarized several other objections that can be made to an interpretation of the elastic properties of active muscle on the basis of results obtained by imposing sinusoidal tension or length changes. But even on the argument we have put forward, it is clear that 'viscosity' as determined by Winton cannot be taken as a direct measure of the muscle's stiffness. In particular, his 'viscosity' cannot be used to compare stiffness during and after stimulation because of the effects which are introduced by active shortening during stimulation. This is undoubtedly the reason for the apparent discrepancy between Winton's 'viscosity' results and the results we have obtained in our stretch experiments.

(*c*) *The series elastic component*

Jewell & Wilkie (1958) have shown that in the frog's sartorius muscle about one-half of the series elastic component is distributed along the muscle's length, i.e. in the contractile apparatus, the rest being located at the ends of the fibres. It seems reasonable to assume that in the *ABRM* as well, part of the series elastic component is located in the contractile apparatus. If, in this muscle, there exist two independent systems in parallel (a paramyosin system, and an actomyosin system) it would be somewhat surprising to find an identical series elastic component in each. But this is precisely what the experiments appear to have shown. Jewell (1959*b*, 1960) found that the series elastic component is the same *during phasic* and *after tonic* stimulation: our results confirm this and demonstrate that this is also true *during tonic* and *during phasic* stimulation (see §3(*h*)).

The finding that the series elastic component is the same in tonic and phasic responses may be interpreted on the independent catch hypothesis as indicating that most of the series elastic component is in series with both the paramyosin system and the actomyosin system (e.g. at the ends of the muscle fibres). If this is so, it follows that at any particular muscle length the sum of active and passive tension will not depend on the amount of passive tension present, as was observed in our experiments (see §3(*k*)). From the experiment shown in figure 16, and from the series elastic curves shown in figure 12, it can be

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calculated that if the explanation according to the independent catch hypothesis is to hold, then no more than 25 % of the muscle's undamped series elasticity can be distributed along the muscle, i.e. 75 % must be at the ends of the muscle. Because this possibility has not been eliminated, the results of the active + passive tension experiment do not in themselves provide conclusive evidence in support of the linkage hypothesis, as we have suggested previously (Lowy & Millman 1959*c*).

The interpretation of this experiment on the linkage hypothesis is straightforward, since active and passive tension are due to the same linkages (see §4(*a*)). Regardless of the location of the series elastic component, the amount of active tension that the muscle can develop on stimulation will depend on the number of linkages that can be formed at sites not occupied by linkages holding passive tension. During maximum contractile activity most of the potential linkages will be formed, and thus the total tension will be almost independent of the amount of initial (passive) tension.

(*d*) *The stretch experiments of Johnson & Twarog* (1960)

Our presentation of the evidence for the linkage hypothesis necessarily involves an assessment of the situation in terms of the independent catch hypothesis. This would be incomplete without discussion of certain stretch experiments which at first sight appear to provide very strong support for the independent catch hypothesis.

Johnson & Twarog (1960) stretched the *ABRM* after tonic (d.c.) and phasic (repetitive) stimulation, and found it to be stiffer after tonic than after phasic stimulation. We have confirmed these results (see §3(*m*)). Johnson & Twarog also compared the amount of tension produced during d.c. stimulation at various muscle lengths with the stiffness as measured at each particular muscle length after cessation of tonic stimulation (i.e. during maintenance of passive tension). In their experiments—before each stretch—the muscle was shortened until tension was reduced to zero and then extended by a fixed amount (3 % l_0), to give a low tension from which to start the actual stretch. Starting from various initial muscle lengths, Johnson & Twarog found that whereas the tension developed during tonic stimulation at the shorter lengths was appreciably less than under the same conditions at the longer muscle lengths, the values obtained for the stiffness when measured as described remained much the same. They concluded that these results support the independent catch hypothesis in that they demonstrate a different dependence on muscle length of the tension-producing (actomyosin), and the tension-maintaining (paramyosin) systems.

In considering their results, it should be pointed out that Johnson & Twarog used d.c. to obtain tonic responses. But as noted in §3(*f*), the response to d.c. is very variable even in the same muscle at the same length. This variability in the d.c. response is in fact confirmed by the results of Johnson & Twarog, which show (their figure 7) that at the same muscle length (e.g. 80 % l_0) very different tensions are obtained in response to d.c. stimulation.

Despite the variability in the d.c. responses, an explanation of the results of Johnson & Twarog is possible in terms of the linkage hypothesis. On this hypothesis the peak tension produced when the muscle is stretched after cessation of tonic stimulation should be related to the number of linkages, and therefore to the amount of passive tension present

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at the time of the stretch. That this is true is suggested by our figure 19, in which peak stretch tension is plotted against 'passive tension'. This figure also demonstrates that peak stretch tension does not necessarily depend on muscle length. In our figure 19, which was derived by measuring the records shown in figure 7 of the paper by Johnson & Twarog (1960), we have estimated the passive tension which would have been present at the time of stretch—had the muscle not been shortened prior to the stretch—by extrapolating the tension decay curves to the time of stretch. This procedure is justified because linkages would not be broken when the muscle was shortened during the maintenance of passive tension (see §4(*b*)).

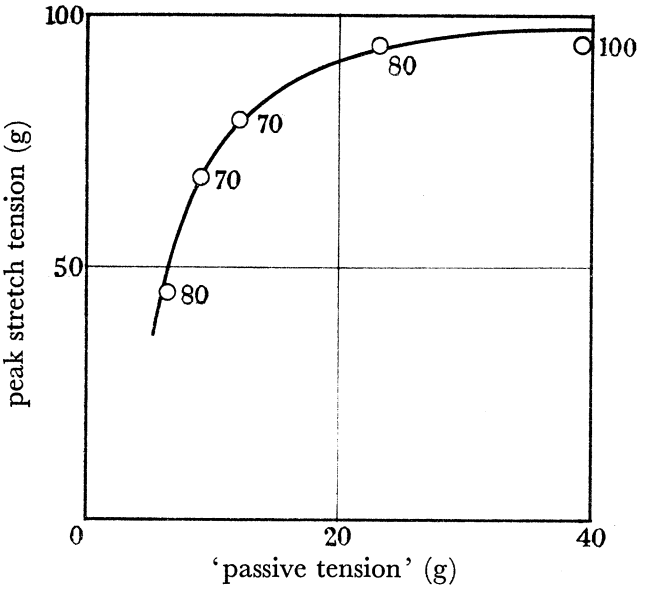

FIGURE 19. Tension produced by stretching the *ABRM* of *Mytilus* after d.c. stimulation. Curve plotted from figure 7 of Johnson & Twarog (1960). The 'passive tension' for each point is the tonic tension that would have been present at the time of stretch, if the muscle had not been shortened prior to the stretch (for full explanation see §4(*d*)). In figure 7 of Johnson & Twarog, five stretches are shown at three different lengths; in the figure above, the length corresponding to the stretch is indicated beside each point as % l_0.

When one takes into account the shape of the stress–strain curve of the undamped series elastic component (see figure 12*b*), and the possible complications involved in stretch experiments (see §4(*k*)), it is not unexpected to find that there is not a linear relationship between linkages and peak stretch tension (see figure 19). Nevertheless our analysis, whilst it does not disprove the case for the independent catch hypothesis, shows that the results reported by Johnson & Twarog are completely compatible with the linkage hypothesis.

(*e*) *Summary of linkage hypothesis*

On the basis of all the evidence summarized above we suggest that contraction in the *ABRM* is produced by linkages of only one type, with one formation rate, but with two different breaking rates, one constant, the other variable. During active shortening most of the linkages are broken at the constant rate, whereas during lengthening or isometric contraction the linkages are broken at the variable rate which is controlled by the concentration of relaxant. In the absence of relaxant the linkages break at a low rate and the

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muscle gives a tonic contraction. When relaxant is present at a concentration sufficient to affect all the reactant sites, the same linkages break at a relatively high rate, and the muscle gives a phasic contraction. In such a mechanism it is not assumed that the relaxant acts directly upon the contractile apparatus, but rather that it affects some reactions intermediate to the breaking of linkages. Thus the concentration of the relaxant could control the rates of these intermediate reactions, and this would allow continuous variation in the speed of tension decay.

Consequences of the linkage hypothesis

(*f*) *Contractile activity and 'active-state'*

In order to consider certain consequences of the linkage hypothesis it will be necessary first to justify our use of the term *contractile activity* in preference to the usual description of muscle activity in terms of 'active-state'.

The presence of 'active-state' is indicated by the ability to develop tension. For the isometric twitch response in the frog's sartorius muscle the decay of 'active-state' has usually been determined by Ritchie's (1954*b*) isometric quick-release method. Here the intensity of 'active-state' present at any instant after the stimulus is measured by the tension redeveloped after a quick release in which tension falls to zero. But in the *ABRM*, the existence of damped elasticity (see §3(*b*)) means that such determinations of 'active-state' reflect not only the presence of active processes but also the effects of inert elastic material. Because of the possibility that measurements of 'active-state' may include effects of inert elastic elements—and this is certainly true in the case of the *ABRM*—we have introduced the concept of *contractile activity*, defined as the ability to shorten and develop tension actively (see §3(*l*)). In terms of the sliding filament hypothesis (cf. Huxley 1960) this would be reflected by the ability to form linkages. Though contractile activity is a parameter that cannot be measured directly it is useful in that it refers only to active processes, and can thus be uniquely related to the contractile mechanism.

It should be pointed out that under certain conditions 'active-state' and contractile activity refer, in practice, to the same thing. Thus during the presence of maximum intensity of 'active-state', linkages will presumably be formed at the highest rate possible at that temperature, i.e. a state of maximum contractile activity will exist.

One of the objects of introducing the concept of contractile activity is to help to give a clearer picture of the process of relaxation. Thus from the above considerations we can now say that when contractile activity has ceased in an isometric contraction below l_0, all the tension present (passive tension) can be considered to be due to the stress exerted by inert elastic elements on linkages which still have to be broken to complete the cycle (cf. Huxley 1960). For a sliding filament system, it may be assumed that active tension is approximately proportional to the number of linkages in action at any instant (cf. A. F. Huxley & Niedergerke 1954; H. E. Huxley 1960). Thus it seems possible that when contractile activity is over, the time course of (passive) tension decay reflects the rate at which linkages break. This situation will be considered first in a tonic contraction of the *ABRM* where the decay of tension can be two orders of magnitude slower than that of contractile activity.

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(*g*) *Relaxation in tonic contractions*

In agreement with Winton (1937) we have observed that following tonic stimulation, the latter part of the isometric tension decay curve is exponential. From the arguments presented above, it is conceivable that this exponential phase of isometric tension decay represents the single process of breaking of linkages, thus giving a rate constant (equal to the reciprocal of the time constant) of about 0·0005/s at 20 °C.

Tension decay does not become exponential until about 10 min after the end of tonic stimulation (figure 11; and figure 18, curve 1). Takahashi (1957, p. 8) concluded from his experiments that 'the inhibitory system responsible for rapid relaxation is also stimulated by (the) chemicals which produce tonic contraction'. Thus the non-exponential form of the curve during this period could be explained by the production, during tonic stimulation, of a certain amount of relaxant. This would mean that some of the linkages would break at a rate higher than the tonic rate. It follows that the rate of tension decay would be *relativity* high immediately after tonic stimulation (whereas it is *relativity* low immediately after phasic stimulation because of the presence of contractile activity, cf. the different shapes of the initial parts of the decay curves after phasic and tonic stimulation in figures 10 and 11 respectively). Thus the characteristic low tonic rate of decay would not become apparent until all the relaxant had been 'used up'. It seems that this is also the state of affairs in an isometric twitch (figure 3; and figure 18, curve 3) where a similar period elapses before tension decays exponentially at the tonic rate. If this explanation is correct, one would expect the relative stiffness of the muscle to be greater some time after the end of tonic stimulation, when all the relaxant has been 'used up'. We have observed this in several experiments (table 3). It should be noted that the effects of the relaxant long outlast contractile activity (see §3(*a*)). Thus the amount of relaxant present after stimulation may be influenced by previous contractions. A similar idea was suggested by Bozler (1930) who found, in the smooth adductor muscle of *Pecten*, that following repetitive stimulation, both stress relaxation and isometric relaxation are speeded up.

These several observations support our explanation for the shape of the tension decay curve before it becomes exponential. As for the exponential part itself, it is possible, though not very likely that even during this phase a small constant amount of relaxant is released. There is no evidence at present which would clear up this point. It has been shown in physiological experiments that the *ABRM* is supplied by both excitatory and 'inhibitory' nerves (van Nieuwenhoven 1947; Hoyle & Lowy 1956; Takahashi 1960; B. Bullard unpublished). If, as we have suggested (Lowy & Millman 1959*b*) the relaxant is released by an 'inhibitory' nervous system, one might succeed in eliminating its effects by experimenting with very small bundles of muscle fibres in the hope that these would lack nerve elements.

Before proceeding to a discussion of relaxation in phasic contractions, it is necessary to consider the effects of 5 *HT* on tonic tension. In §3(*f*) an experiment was described in which 5 *HT* was applied to a muscle holding passive tension several minutes after the ability to develop active tension was over, i.e. when none of the linkages present were being broken at the high phasic rate. The observed effect of 5 *HT* was the conversion of

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the tension decay rate from the low tonic value to the characteristic high phasic value. This is also shown by the twitch, where the time course of tension decay is changed from the tonic to the phasic rate in the presence of 5 *HT* (see figure 10). These observations suggest to us that the same forces are involved in tonic and phasic tension decay; an explanation which follows from the linkage hypothesis, according to which the same linkages are responsible for both tonic and phasic tension.

The effects of 5 *HT* described above can also be accounted for on the independent catch hypothesis, if one assumes that relaxation under all conditions is due to the paramyosin system. This would mean that an actomyosin system is the shortening system, but that during phasic as well as tonic stimulation the paramyosin system is also brought into action (see also Johnson & Twarog 1960). The paramyosin system would be 'plasticized'. in the presence of 5 *HT* and this would give the phasic decay rate; whereas in the absence of 5 *HT* the paramyosin system would be rigid, and tension therefore would decay slowly. The difficulty with this explanation is that it also has to be assumed that release of the muscle during tonic stimulation 'plasticizes' the paramyosin system (see §4(*b*)).

(*h*) *Relaxation in phasic contractions*

From the findings that in the absence of contractile activity the tension decay after repetitive stimulation is exponential, and that this rate of decay is affected very little by 5 *HT* (§3(*e*)), it appears that the decay rate under these conditions represents the maximum possible linkage breaking rate. The close agreement in the shape of the decay curves and in the magnitude of the decay constants between the response to repetitive stimulation and a twitch in the presence of 5 *HT* (figure 9), strongly suggests that these responses are basically identical. We have also shown that by the addition of 5 *HT*, one can convert the tonic tension decay rate to the phasic one (§3(*f*)). Thus it would appear that depending on the concentration of 5 *HT* present, the linkage breaking constant in the *ABRM* can be varied from 0·3/s, representing a pure phasic contraction, to 0·0005/s, representing a pure tonic contraction.

It follows from these considerations that the only difference between tonic and phasic responses is that in the latter, the linkages break at a high rate due to the presence of a 5 *HT*-like relaxant, which is released by repetitive stimulation. Small amounts of relaxant are apparently released by all external stimuli, even in the case of a single shock when the response is largely tonic (see §4(*g*)). Our experiments show that at frequencies of stimulation greater than about 4 shocks per second enough relaxant is produced to give a completely phasic response.

There is good evidence for the identification of 5 *HT* as the natural relaxant in the *ABRM* (cf. Twarog 1954, 1960). It has been shown (Holgate & Cambridge 1958) that concentrations of 5 *HT* as low as 10^{-9} g/ml. have a relaxing effect on the muscle. Furthermore, 5 *HT* has been identified in *Mytilus* ganglia (J. H. Welsh & M. Moorhead, unpublished results quoted by Twarog 1960), and 5 *HT* destruction in the *ABRM* by enzyme activity has also been demonstrated (Blaschko & Milton 1959). The problem now is to demonstrate how 5 *HT* produces the relaxing effect in the *ABRM*.

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(i) The mechanism of phasic contraction

We have stated in a preliminary communication (Lowy & Millman 1959*a*) that the tension plateau produced by repetitive stimulation at a frequency of 4 to 10/s is maintained by asynchronous discharges from spontaneously active muscle and/or nerve elements. These results are described in detail in §3(*d*). Similar observations were made by Fletcher (1937*b*) who stimulated the muscle for 12 min with condenser discharges of alternating polarity at 30/s. His electrical and mechanical records show relatively large fluctuations which, as in our experiments, are not related to the stimuli in any obvious way.

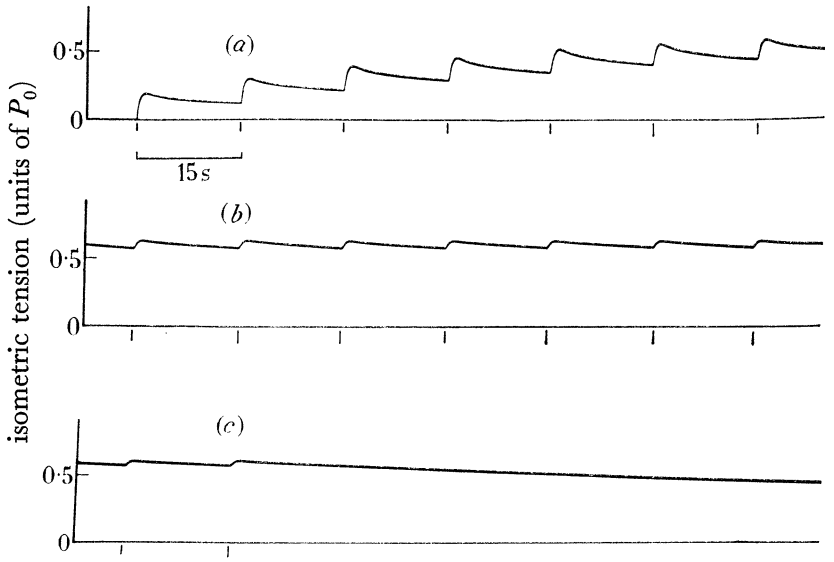

FIGURE 20. Development and maintenance of isometric tonic tension in the *ABRM* of *Mytilus*, stimulated with a single shock once every 15 s. Stimuli indicated below the tension records. (*a*) Development of tonic tension; (*b*) same contraction, maintenance of a level of tension after stimulation for 7 min; (*c*) same contraction, after stimulation for 10 min, showing the slow decay of tension when stimulation is stopped. Temperature = 21 °C, length = 3·0 cm, weight = 30 mg, P_0 = 10·6 kg/cm^2.

Jewell (1960) has argued that in the *ABRM* repetitive stimulation produces a response like a tetanus in the frog's sartorius muscle. He found that in a series of phasic contractions in the *ABRM* the isometric myograms were identical in every detail. Other workers (Fletcher 1937*a*; Prosser, Curtis & Travis 1951; Schmandt & Sleator 1955) have observed that repetitive stimuli at a frequency below about 10/s produce a steady level of depolarization with superimposed action potentials, which bear a one-to-one relation to the stimuli. Taking the second point, our observations agree with those of the other workers about what happens during the first 10 to 20 s after the beginning of stimulation (§3(*d*)). As regards the first point, it should be noticed that the irregular fluctuations in tension level which we observed do not become detectable until at least 20 s after the beginning of stimulation, and even then they are very small, i.e. only about 1 % of the total tension developed. Jewell did not stimulate the muscle for longer than 30 s, and it is therefore not surprising that he found very little variation in his series of phasic responses. He may well be right in saying that during the first 30 s or so after the beginning of repetitive stimula-

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tion the response of the *ABRM* is like a tetanus in the frog's sartorius muscle. But after that our experiments show that tension declines, and the character of the response probably changes.

(*j*) *The mechanism of tonic contraction*

On the basis of our explanation of the response to a single stimulus it is now possible to suggest an experimental procedure for the maintenance of a steady high level of tension, which very likely corresponds to the mechanism of tonic contraction *in vivo*. Single shocks, spaced at intervals of 10 to 30 s, should enable a large amount of tonic tension to be developed and maintained with very little energy expenditure. By stimulating in this manner, we found that the *ABRM* can produce tension up to 75 % P_0, and after reaching this level of tension, can maintain it steadily for several hours (figure 20; and Lowy & Millman 1959*b*).

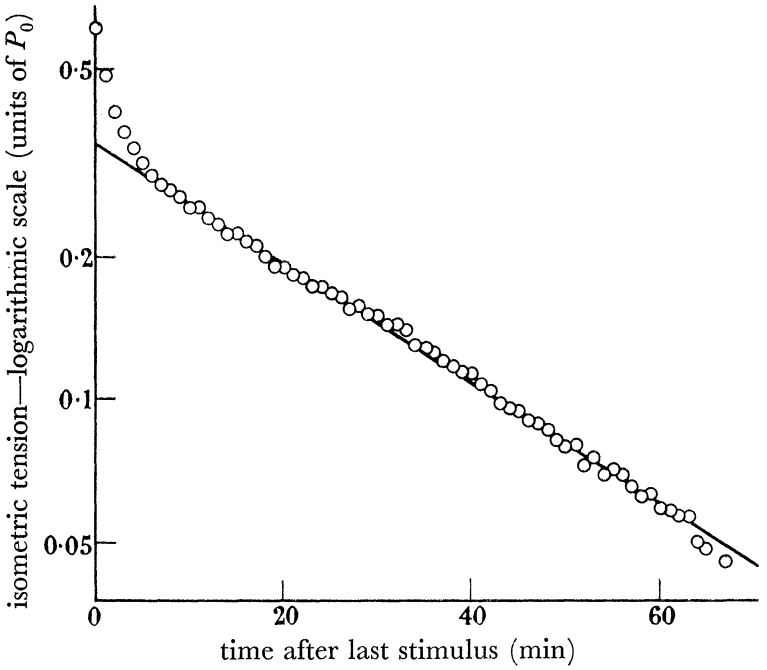

FIGURE 21. Decay of isometric tonic tension in the *ABRM* of *Mytilus*, produced by applying single stimuli at 15 s intervals. Same contraction as shown in figure 20. Time constant of exponential part of tension decay = 34 min.

After such stimulation tension decays slowly, as in a tonic response, the decay curve again becoming exponential about 10 min after the end of stimulation (figure 21). Stimulation in such experiments has been continued for periods up to 20 h, at which time tension has usually dropped to a level corresponding to about 15 % P_0. After such a prolonged contraction, when the muscle is stimulated with shocks at 10/s, the same maximum tension is obtained as that which could be developed at the beginning of the experiment. Evidently the ability for active tension production has not diminished. It seems rather that the conditions of stimulation required for the maintenance of a tonic contraction change with time. Given suitable activation, it seems very likely that the muscle could be made to hold a high level of tension until its energy supply was exhausted.

We consider that the method of stimulation described above closely resembles what may happen during a prolonged tonic contraction of the muscle *in vivo* (see also Jewell 1959*b*),

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and propose to call this explanation of tonic contraction the *intermittent activation mechanism*. An observation which suggests the existence of just such a 'built-in' activation mechanism comes from experiments in which the unstimulated *ABRM* was stretched above l_0. This often produces a tonic contraction maintained by 'spontaneous' bursts of activity which occur about once every 10 s (figure 15). The individual responses, which resemble twitches, are accompanied by action potentials of the same magnitude as those accompanying normal twitches. An intermittent activation mechanism is also suggested by recordings of muscle action potentials during tonic contraction in the (externally) denervated posterior adductor muscle of *Mytilus*, where intermittent bursts of activity have been observed throughout the period for which the shells are kept closed (Lowy 1953, 1955). For the posterior adductor, this involves holding a tension of about 0·8 kg/cm^2, which is the tension required to counteract the force exerted by the elastic ligament at the hinge of the shell (cf. Marceau 1909). In our experiments the *ABRM* could generate and maintain almost ten times that tension. It seems unlikely that such high tensions would continuously be required *in vivo*, where the *ABRM* is one of several retractor muscles which form the apparatus by which the animal (average weight 25 g) suspends itself by the byssus threads from a solid substratum. Such high tensions might be required, however, when the animal is exposed to violent wave action.

(*k*) *Effects observed in stretch experiments*

On the linkage hypothesis it is expected that a large part of the tension produced during stretch would be due to the resistance offered by linkages. This is in agreement with the stretch experiments, which show: (*a*) that in the resting muscle where few linkages are present (as indicated by a low initial tension) a small stretch tension is produced, and (*b*) that in the presence of slow-breaking tonic linkages more stretch tension is produced than in the presence of fast-breaking phasic linkages, regardless of whether the phasic linkages are generated by phasic stimulation or by tonic stimulation in the presence of 5 *HT* (table 3).

If stretch tension during contraction is due to linkages, the question arises as to why a higher value can be obtained after tonic stimulation (when presumably fewer linkages are present) than during such stimulation (e.g. figure 17*a*, *b*). A possible answer is that the factor which limits stretch tension under both these conditions is not the presence or absence of linkages, but structural constraints within the muscle which are not directly affected by stimulation. This idea is suggested by the observation (see §3(*m*)) that although the sum of initial tension and stretch tension (i.e. total tension) is always lower after than during tonic stimulation, examination of the records suggests that both stretch curves are approaching the same limiting value of total tension (figure 17*a*, *b*). Since in a stretch during tonic contraction the total tension often exceeds P_0, it is likely that the breaking stress of the muscle is approached, and some of the muscle elements may be extended beyond their reversible limits. This is indicated by the finding that after a stretch during tonic contraction in which a very high total tension was reached, the muscle usually developed less active tension in subsequent contractions.

We have already mentioned (§4(*g*)) another factor which would affect the stiffness of the muscle during and immediately after tonic stimulation, namely the production of small

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amounts of relaxant during such stimulation. This would mean that linkages would be broken more easily by a stretch during than after tonic stimulation, and hence the relative stiffness would be greater after such stimulation.

(*l*) *Stress relaxation*

Bozler (1930) who worked with the smooth pharynx retractor muscle of *Helix* and with the smooth part of the adductor of *Pecten*, stated that the decay of tension after a relatively rapid stretch of the resting muscle (stress relaxation), and the decay of isometric tension after electrical stimulation (isometric relaxation), both follow the same exponential time course. He also found that in the *Helix* muscle the decay rates of both stress relaxation and isometric relaxation are similarly affected by variations in temperature and CO_2 concentration; and that in the *Pecten* muscle both kinds of relaxation are similarly speeded up following repetitive stimulation. Accordingly, he suggested that 'in the beginning of relaxation, the (smooth) muscle is in the same physical state as if the resting muscle had been stretched' (Bozler 1936, p. 429).

If what is meant by 'in the beginning of relaxation' is more precisely defined, much the same conclusion follows from our interpretation of the stress relaxation results in the *ABRM*. We assume that following tonic stimulation, passive tension is present due to the stress exerted by inert elastic elements on linkages which break at the low tonic rate (see §4(*f*)). We also find that the third (or slowest) component of stress relaxation has the same decay rate as the latter part of the tonic tension decay curve, and thus presumably represents the breaking of these same tonic linkages (see §3(*n*)). It is possible that the breaking of linkages at the high phasic rate is represented by the second component of stress relaxation. This component is the slowest one present in the 5 *HT*-treated muscle and has approximately the same decay rate as the exponential part of tension decay in a phasic contraction (see §3(*e*)).

From these results it appears that Bozler's suggestion also applies to the *ABRM*. For the *Helix* muscle, Abbott & Lowy (1958*b*) have cast doubt on Bozler's interpretation because they found that here stress relaxation could be represented as the sum of two exponential curves, whereas the time course of tension decay following repetitive stimulation followed a single exponential curve. It should be noted, however, that Bozler did not obtain agreement between his two curves until peak tension had fallen by about one-third, and it is during this first part of tension decay that the faster of the two components of Abbott & Lowy would have the greatest effect. It seems possible that the slower stress relaxation component of Abbott & Lowy may in fact have the same decay rate as the exponential part of isometric relaxation: i.e. as in the *ABRM*, during the latter part of tension fall the two curves may be identical. This idea is not as unlikely as would appear from examination of figure 5 of Abbott & Lowy (1958*b*), because a comparatively small shift in the zero position of tension in such a semi-logarithmic diagram can cause a considerable change in the slope of the curve: this is especially likely to occur at low tension values.

We therefore conclude that provided equivalent situations are considered, Bozler was correct in saying that the physical state of the smooth muscles investigated is the same during stress relaxation as during isometric relaxation.

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(*m*) *Conclusion*

On the available evidence, it is not yet possible to make a final decision between the independent catch hypothesis and the linkage hypothesis. The independent catch hypothesis explains tonic contraction in muscles like the *ABRM* by assuming that one of two functionally independent systems becomes rigid; whereas the linkage hypothesis postulates the existence of only one system, similar to the tension-producing system in vertebrate striated muscle, the *ABRM* system being specialized in that under certain conditions tension decays extremely slowly. On balance, we favour the linkage hypothesis because we consider that it offers a simpler explanation for the contractile mechanism in muscles like the *ABRM* than does the independent catch hypothesis.

The linkage hypothesis cannot at present account for the observation (Rüegg 1961 *a*, *b*) that agents which abolish 'active' tension in actomyosin systems do not completely relax glycerol-extracted or 'surviving' muscle fibres from the *ABRM*, i.e. there is a 'tension remnant'. It has yet to be shown, however, that this 'tension remnant' is the same as tonic tension in the living muscle. Furthermore, the independent catch hypothesis cannot straightforwardly explain certain observations on the living *ABRM*, e.g. that the shortening speed at zero load is identical during tonic and phasic stimulation, although the system which is responsible for tonic tension is in action during tonic stimulation.

We wish to express our thanks to Professor J. T. Randall, F.R.S. (now Sir John Randall), for the encouragement he has given to this work; and to Dr J. Hanson and Dr H. E. Huxley, F.R.S., for helpful discussion and criticism during the preparation of the paper. We are grateful to Miss B. Bullard and Mr G. L. Lawrence for technical assistance. One of us (B.M.M.) is indebted to Imperial Oil Limited of Canada and to the National Research Council of Canada for financial support. Part of the electronic apparatus was purchased with a grant from the Royal Society to one of us (J. L.).

[*Note added in proof*, 9 *November* 1962.] Two important contributions to our knowledge of the contractile mechanism of the *ABRM* have appeared since this paper was written. Further experiments in support of the independent catch hypothesis have been presented by W. H. Johnson, together with a detailed discussion of the conflict between this hypothesis and the linkage hypothesis (*Physiol. Rev.* **42**, Suppl. 5, 113).

Support for the linkage hypothesis comes from investigations by J. V. Howarth (*J. Mar. biol. Ass. U.K.* **42**, 727) on the thermoelastic properties of the *ABRM*. He found that during tonic stimulation with *ACh* the thermoelastic heat is similar in sign and magnitude to that found in active amphibian striated muscle. When *ACh* is washed out from the *ABRM* the thermoelastic heat remains initially unchanged, but gradually reverts to that characteristic of the resting muscle as tension falls. This indicates that the contractile apparatus of the active *ABRM* has a cross-linked structure which persists during the maintenance of tonic tension and gradually disappears as tensions falls.

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