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THE NATURE OF THE PHASIC AND THE TONIC RESPONSES OF THE ANTERIOR BYSSAL RETRACTOR MUSCLE OF *MYTILUS*

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Since Winton (1934) first introduced the anterior byssal retractor muscle (ABRM) of *Mytilus* into muscle physiology, various workers have found that it provides a particularly suitable preparation for use in studying the physiology of lamellibranch muscle. Its virtues are, first, that it is made up of a homogeneous population of muscle fibres, each of which probably extends from one end of the muscle to the other; secondly, that it can easily be isolated intact for experiments *in vitro*. Various types of stimuli will make the muscle contract, but the time course of the relaxation that follows depends on the nature of the stimulus. This has provided a convenient basis for dividing the responses of the ABRM into two general categories; 'phasic' responses, in which the relaxation is complete within about 30 sec, and 'tonic' responses, in which the corresponding degree of relaxation may take from several minutes to several hours. In general, acetylcholine and direct-current stimulation produce tonic responses, and most other forms of electrical stimuli produce phasic responses.

In the interpretations that have been given of the behaviour of the ABRM during phasic and tonic responses there has been an exacerbation of the traditional controversy that exists over the nature of the tonic contraction produced by many lamellibranch muscles. The controversy arose at the beginning of the century when there was disagreement over the suggestions made by certain workers (for references, see Grützner, 1904; Marceau, 1909) that this type of muscle contains a special (passive) mechanism which enables it to maintain contractions economically for long periods. The concept of a 'catch' mechanism was first considered in detail by Grützner (1904), and it was greatly strengthened by von Uexküll's (1912) classical experiments on the adductor mechanism of *Pecten*. However, other workers rejected the idea that a special mechanism is necessary for the maintenance of a tonic contraction. It has been argued that in view of the intrinsic slowness of this type of muscle,

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considerations of the economics of the situation do not exclude the possibility that the tonic contraction is tetanic in nature (see Ritchie, 1928; Bayliss, 1928).

These two points of view are usually referred to as the 'catch-mechanism' and 'tetanus' hypotheses, respectively, and it has commonly been supposed by those engaged in the controversy that the two must necessarily be mutually exclusive, and that one or other hypothesis must be correct. Criteria for distinguishing between them have been based on three quite different features of muscular contraction; and interest has been concentrated on (i) the type of activity in the excitatory system, (ii) the energy cost of the contraction, and (iii) the changes in the mechanical state of the muscle. The fact that the tonic response is accompanied by (*a*) a rise in the rate of heat production of the muscle (Abbott & Lowy, 1958*a*), and (*b*) a prolonged increase in the rate of firing of spontaneously active elements (Hoyle & Lowy, 1956) has been claimed to support the tetanus hypothesis. On the other hand, the fact that (*a*) the muscle shows a high 'viscosity' after stimulation with direct current (Winton, 1937; van Nieuwenhoven, 1947; Johnson, 1954), and (*b*) that relaxation is much slower than the repolarization of the fibre membranes after the muscle has been treated with acetylcholine (Twarog, 1954) has been claimed to support the catch-mechanism hypothesis. Thus the findings themselves seem to show that the behaviour of the ABRM in a tonic response does not exactly follow the pattern predicted by either hypothesis, and it now seems as if both may have outlived their usefulness.

The additional results that have recently been reported in preliminary communications by Johnson (1958), Lowy & Millman (1959), and Jewell (1959) also support this view, and they show that there has been some overlap in the recent work that has been done on the ABRM by the different workers. Fortunately, there appears to be general agreement over the experimental findings, though there is still some disagreement over certain points in their interpretation. In the present paper it will be shown, by means of release experiments, that two different states of contraction can be produced by the ABRM. It will be seen later that this finding can be used to give reasonable interpretations of the nature of the tonic response and of the relationship between the phasic and tonic responses, which do not involve strict adherence to either of the traditional hypotheses.

METHODS

Dissection

Specimens of *Mytilus galloprovincialis*, which were obtained from Padstow (Hepper, 1957), were used in all the experiments described in this paper. The two valves of the shell were forced apart with a screwdriver, and the posterior adductor muscle was severed. The byssal threads were cut off and all the soft viscera, including the foot, were removed. The anterior and posterior byssal retractor muscles on one side of the animal were then severed close to their insertion into the byssal gland, and that half of the animal was discarded; the remaining half then had the

appearance shown in Fig. 1*a*. Two holes were made about 1 mm from the dorsal edge of the shell, one at each end of the insertion of the ABRM (see Fig. 1*a*). The rectangular part of the shell around the insertion of the ABRM was then cut out with a hack-saw, and the PBRM was severed as shown by the interrupted line in Fig. 1*a*. The thread that had been tied around the base of the byssal stem was later used to attach it to the stainless-steel wire that connected the muscle to the recording apparatus.

Mounting

The rectangular piece of shell was then securely fixed to the L-shaped Perspex muscle holder with 36 S.W.G. (0·02 mm) stainless-steel wire, as is shown in Fig. 1*b–d*. It is in the mounting of the preparation that the present technique differs appreciably from those used by other workers in this field. It was devised to solve the problem of arranging the isolated muscle so that the stress distribution is uniform over its cross-section. When the ABRM is *in situ* (Fig. 1*a*), it can be seen that the byssal stem makes an acute angle with the long axis of the muscle. However, when the isolated muscle is placed under stress (either by stretching it or by stimulation under isometric

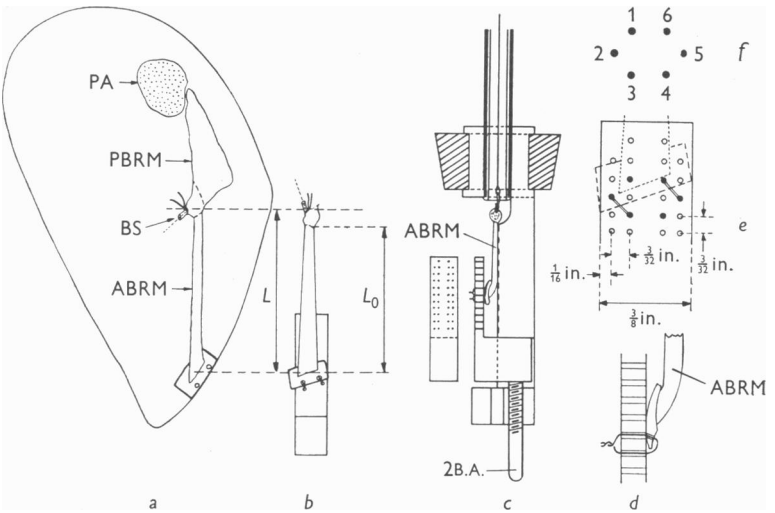

Fig. 1. Method of mounting the ABRM on the multi-electrode assembly. (*a*) View of the half-shell showing the anterior (ABRM) and posterior (PBRM) byssal retractor muscles, the posterior adductor (PA), and the byssal stem (BS). The dorsal (hinge) edge of the shell is on the right-hand side of the diagram. The PBRM was severed along the interrupted line at the byssal end of the muscle. (*b*) View of the electrode surface of the mounted preparation. L = length of the preparation *in situ*; L_0 = body length of the muscle. (*c*) Longitudinal section through the multi-electrode assembly (see Hill, 1949), showing the method of clamping the L-shaped muscle holder in place by means of a 2 B.A. screw. (*d*) Diagram to illustrate the method of fixing the preparation to the muscle holder with 36 S.W.G. wire. (*e*) Diagram to show the spacing between the holes in the muscle holder: measurements marked in fractions of inch; 1/16 in. = 1·6 mm. There is a set of holes for each muscle length; the set used in this particular case has been darkened. (*f*) A similar diagram in which these holes have been numbered.

conditions), the orientation of the structures at its byssal end changes, because the angle that the byssal stem makes with the long axis of the muscle increases considerably (see Fig. 1*b*). The result is that the fibres along the ventral edge of the muscle bear more of the stress than those on the dorsal edge, if the preparation has been set up so that the position of the shell (relative to the long

axis of the muscle) is the same as it was *in situ*. By rotating the piece of shell into the position shown in Fig. 1*b*, it is possible to bring the fibres under uniform stress. The correct position was determined by examining the muscle under a low-power microscope during the application of gentle stretches; when the fibres were unstretched, the surface of the muscle had a curious wrinkled appearance, which disappeared as soon as the muscle was slightly stretched. The position of the shell could be adjusted carefully so that the wrinkles disappeared simultaneously over the entire surface of the muscle, showing that the effect of the stretch was being distributed uniformly. Several muscles were examined in this way, and it was found that the required adjustment was always about the same; a standard method was therefore adopted for fixing the pieces of shell on all preparations in the same position relative to the long axis of the muscle. This consisted of drilling holes in the muscle holder so that the two ends of the shell could be fixed as is shown in Fig. 1*d*. For a variety of reasons it was desirable to have the preparation arranged so that the byssal gland lay in the gully above the top electrode on the standard (frog sartorius) type of multi-electrode assembly (see Fig. 1*c*). As the position of the muscle holder was fixed, this meant that a number of sets of holes were required so that muscles of different lengths could be used; the arrangement of the holes was as shown in Fig. 1*e*. The set of holes corresponding to a particular muscle length is shown in Fig 1*e* and *f*; if the ABRM came from the left-hand side of the animal as in Fig. 1*a*, then the wires passed through holes (2–3) and (5–6); if it came from the other side, then they passed through holes (1–2) and (4–5). In this way, all muscles were mounted so that the distribution of stress over their cross-sections was approximately uniform.

The horizontal limb of the muscle holder was clamped against the underside of the electrode block by means of a 2 B.A. Perspex screw and its position was adjusted so that the whole of the muscle was in contact with the electrodes (Fig. 1*c*). The byssal stem was tied to the stainless-steel connexion, as described by Jewell & Wilkie (1958), and the length of the preparation was adjusted until it was the same as it had been *in situ* (L, Fig. 1*a*); the body length of the muscle (L_0, Fig. 1*b*) was then measured. The jacket around the electrode assembly contained oxygenated sea water at room temperature. This was automatically run out 30 sec before electrical stimuli were applied to the muscle.

Recording apparatus

The mechanical recording system consisted of a Levin-Wyman ergometer and a light isotonic lever, coupled together as shown in Fig. 2. As the tensions produced by the ABRM were excessively large for direct transmission to the transducer valve (RCA 5734), the muscle was attached to the isotonic lever in the position shown; this gave a 2:1 reduction in the force recorded by the transducer. The isotonic lever was fitted with a photo-electric recording system for measuring movement, so that simultaneous records could be made of the changes in length and tension in the muscle during various types of contraction. The outputs of the transducer and photocell were usually fed into the two channels of a Cossor 1049 oscilloscope, and the photographic records were made with a 35 mm cine-camera. However, when greater accuracy was required, quarter-plate records were made from a larger single-beam tube. The mechanical recording system was used in four different ways in these experiments:

Isometric responses. The catch (C) was released and the ergometer arm was held in the required position by means of the stop (H). When the weights (N) were removed from the isotonic lever, the transducer recorded half the tension in the muscle. Calibrations could be made without disturbing the connexion to the muscle, by hanging weights at the tip of the isotonic lever.

Isotonic responses. The coupling link (Q) was removed and the isotonic lever was then used in the normal way for recording isotonic contractions. Loads (N) were hung on the lever by means of the compliant support (G), and the length of the muscle could be adjusted by means of the after-load stop (B), which was also used for calibrating the length-recording system (1 revolution equalled 0·5 mm).

Controlled releases. The transducer was held in the 'up' position by engaging the catch (C) in the end of the ergometer arm. When the key (K) was opened at a pre-set time during the contraction, the catch (C) was released and the ergometer arm moved the transducer downwards

through a distance determined by the position of stop (*H*), and at a speed governed by the strength of the spring (*S*) and the setting of the needle valve in the dashpot (*D*). The type of measurement that was made depended on the system used to display the length and tension changes in the muscle. If they were displayed on a slow time base, studies could be made of the redevelopment of tension after releasing the muscle at different speeds and by different amounts. If they were displayed on a fast time base (Hill, 1950) or plotted against one another on a single-beam tube (Jewell & Wilkie, 1958), measurements could be made of the load-extension curve of the series elastic elements by means of the controlled-release method.

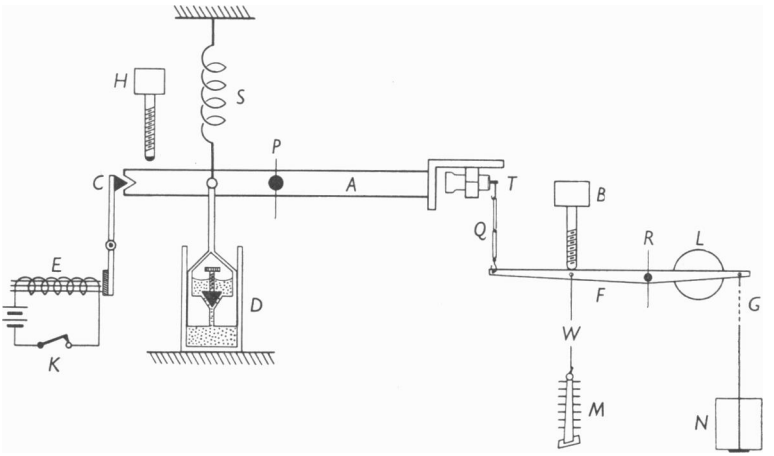

Fig. 2. Arrangement of mechanical recording apparatus. The muscle (*M*) lies on the multi-electrode assembly, and a stainless-steel wire (*W*) connects its byssal attachment to the isotonic lever (*F*). The lever is pivoted on ball bearings at *R*, and its movements are recorded by means of the photo-electric system (*L*). Weights (*N*) are hung on the lever through a compliant support (*G*) and the length that the muscle adopts is determined by the position of the adjustable stop (*B*). A flexible, but inelastic, connexion (*Q*) can be used to attach the isotonic lever to the transducer valve (*T*), which is mounted at the end of the moving arm (*A*) of a Levin-Wyman ergometer.

Isotonic releases. The needle valve in the dashpot (*D*) was opened as far as possible, so that the ergometer arm could move at its maximum velocity, and the transducer was held in the 'up' position by means of the catch (*C*). Weights (*N*) were hung on the lever to produce the required load on the muscle, and the after-load stop (*B*) was adjusted to take up any slack in the coupling (*Q*). At first the contraction was isometric and the transducer recorded the tension in the muscle as soon as this exceeded the value of the load on the muscle. When the key (*K*) was opened at a pre-set time during the contraction, the catch (*C*) was withdrawn and the transducer was moved downwards at a velocity which greatly exceeded the maximum velocity of active shortening of the muscle, with the result that the coupling link (*Q*) went slack. The conditions in the muscle were then isotonic and it shortened freely against the constant load produced by the weights (*N*).

RESULTS

Production of phasic and tonic responses

Some of the untidy features of the ABRM are revealed when the relationship between the tension produced by the muscle and the strength of the stimulus (strength–response curve) is determined for different types of stimuli. These

show, first, that it is extremely difficult to make this muscle give a maximal response, and secondly, that the maximum tension which can be obtained in a phasic response is only about 70% of that obtained in a tonic response. Presumably these findings reflect inadequacies in the methods that are now in use for stimulating the muscle. They have certainly meant that the choice of suitable stimulus parameters for producing phasic and tonic responses has been somewhat arbitrary.

Phasic responses

The ABRM was stimulated on a multi-electrode assembly, which was connected so that there were alternative anodes and cathodes. The responses that were produced by stimulating the muscles with alternating condenser shocks at 10/sec (see, for example, Figs. 3*a* and 7*a*) were larger than any that could be obtained by using square pulses or alternating currents (at frequencies from 10 to 1000 c/s). When the strength–response curve for this type of stimulation was determined, it was found that as the stimulus strength was increased the slope of the curve decreased, but a plateau (corresponding to supramaximal stimulus strengths) was never actually attained, because the muscles began to show signs of damage; for instance, relaxation became slower and the responses to repeated similar stimuli decreased in size. A similar strength–response curve was reported for the muscle action potential by Schmant & Sleator (1956). By using a stimulus the strength of which was about 10% less than that required to produce detectable slowing of relaxation, it was possible to obtain reproducible responses in which the tensions produced were probably within about 90% of the maximum value possible under these conditions of stimulation. The voltage required for this purpose depended on the season; with a time constant of about 6 msec (capacity, 3 μF), the muscles needed 1·7–2·6 V in the winter and spring, and 1·0–1·5 V in the summer.

Alternating stimuli were used because shocks of this strength produced a good deal of polarization if they were unidirectional; and they were applied at 10/sec because the rate of rise of tension was found to be maximum at this frequency. The highest tension produced by any muscle in an isometric tetanus corresponded to a stress of 10·7 kg wt./cm^2. It was assumed that muscles which gave less than 6 kg wt./cm^2 had been damaged in the dissection, and these were therefore rejected.

Tonic responses

Acetylcholine (ACh) was used in preference to direct current (d.c.) for producing tonic responses, because when the latter was used with the multi-electrode assembly, (i) tensions above about 8 kg wt./cm^2 could not be obtained without damaging the muscle, and (ii) the contractions were very

non-uniform along the length of the muscle fibres, because the responses to d.c. are cathodal (see Fletcher, 1937*c*).

When solutions containing more than 10 μg ACh/ml. were used to produce tonic responses, the tensions developed were invariably greater than those produced in phasic responses, even when shocks of damaging strength were used (compare Fig. 3*a* and *b*). All the tonic responses described in this paper were produced by immersing the muscles in solutions containing 100 μg ACh/ml. in sea water; the tensions produced when different muscles were treated in this way varied from 11·0 to 12·5 kg wt./cm^2. However, this was not a maximal response, because greater tensions could be produced by using stronger solutions (one muscle developed 13·7 kg wt./cm^2 when it was treated with 1 mg ACh/ml.). After the muscle had been immersed in the oxygenated solution for 2 min (or for longer if the tension had not reached a plateau by that time), it was thoroughly washed in several changes of sea water to ensure that the traces of ACh that remained on the muscle were reduced to subthreshold concentrations. When the ACh solution was replaced by sea water on the first occasion, the contraction was not usually well maintained. However, the decay of tension became progressively slower after each immersion in ACh, and on the third or fourth occasion it usually fell to about 70% during the first 10 min in sea water, and extremely slowly thereafter; measurements could then be made under reasonably steady conditions. The tonic responses produced by ACh and by d.c. stimulation are fully reversible, and they can be terminated at any stage by stimulating the muscle with repetitive shocks or by treating it with 5-hydroxytryptamine (see Fletcher, 1937*b*; Twarog, 1954; Cambridge & Holgate, 1959).

In experiments on the ABRM, it is important to stimulate the muscle at regular intervals because it exhibits a marked 'staircase' effect, even in tetanic responses (see Winton, 1937). During experiments that involved a series of tetani, fatigue was minimized by allowing the muscle a 30 sec rest for every second of stimulation; as tetanic contractions lasting 20 sec were frequently needed, this meant that the intervals between successive contractions were rather long (i.e. 10 min), but more frequent stimulation invariably led to deterioration of the muscle. In experiments involving tonic responses, the intervals between the periods of stimulation were largely determined by the effects of the previous stimulus.

Redevelopment of tension after a quick release

Phasic response

When a quick release is made during an isometric tetanic contraction, the behaviour of the ABRM bears a strong qualitative resemblance to that of other muscles, such as the frog sartorius (Gasser & Hill, 1924), the body-wall muscle of *Holothuria nigra* (Hill, 1926), and the retractor penis muscle of

the tortoise (Goodall, 1957). If the release is large enough to discharge the tension in the series elastic elements, and if the speed of release exceeds the maximum velocity of active shortening of the muscle, the tension falls to zero during the release and then redevelops to a final level that is characteristic of the new length.

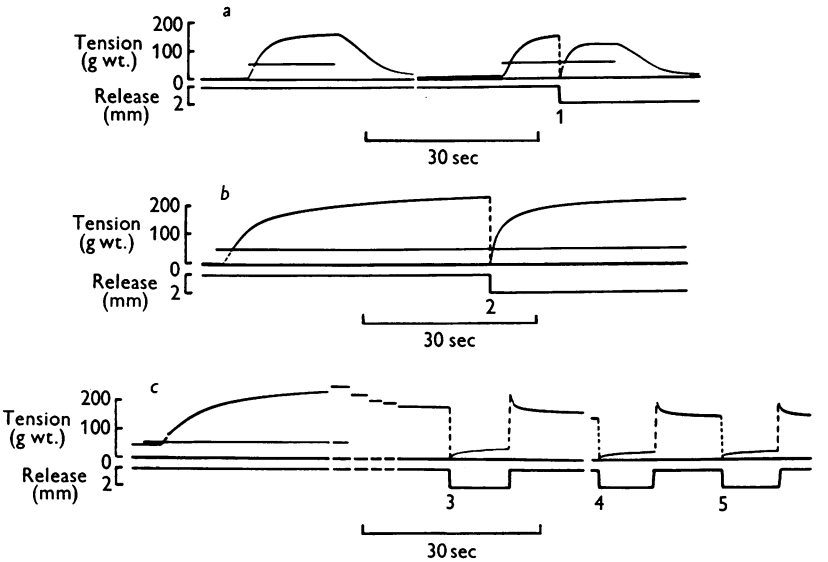

Fig. 3. Redevelopment of tension after quick releases made during phasic and tonic responses. ABRM: L_0 = 30 mm, weight = 65 mg; temp. 18° C. 35 mm records: one trace shows the tension of the muscle, the other shows when the muscle was being stimulated; the records have been retouched where they were too faint to reproduce satisfactorily. Lines have been added to show zero tension and the length changes in the muscle. Releases: from $L_0 + 1$ mm to $L_0 - 1$ mm at 50 mm/sec. (*a*) Release during a phasic response. The first record shows an isometric tetanus, and the second record shows the effect of a release 10 sec after the onset of stimulation in a 20 sec tetanus (Release 1). (*b*) Release during stimulation in a tonic response; 50 sec after immersion in ACh solution (Release 2). (*c*) Releases after stimulation in a tonic response. The muscle was first treated with ACh, and the four short bars show the tension levels, at the end of stimulation and then 3, 6, and 9 min after the ACh was removed. It was subsequently released to $L_0 - 1$ mm, and then re-stretched to $L_0 + 1$ mm, 12 min (Release 3), 14 min (Release 4), and 14 min 20 sec (Release 5) after the ACh was removed. The following results were obtained by analysing the records:

		Tension (g wt.)	
Release	$L_0 + 1$ mm (Before release)	$L_0 - 1$ mm (10 sec after release)	$L_0 + 1$ mm (10 sec after re-stretch)
1	150	132 (82)	—
2	244	207 (84·8)	—
3	187	38 (20·3)	172
4	151	27 (17·9)	157
5	155	28 (18·3)	155

The figures in brackets give the amount of tension redevelopment expressed as a percentage of the tension immediately before the release.

Figure 3*a* shows a typical record of the tension changes that occurred in an ABRM when it was released from L_0+1 mm to L_0-1 mm at 50 mm/sec, 10 sec after the onset of stimulation in a 20-sec tetanus. The tension redeveloped to 82% of the value recorded immediately before the release. The amount of tension redevelopment would be expected to depend simply on the tension–length relationship of the muscle, unless the ABRM resembles certain insect muscles in which the contractile mechanism appears to be partially inactivated by a sudden change in length (Pringle, 1954). To exclude this possibility comparisons were made between the tensions redeveloped at different lengths and the tensions developed in straightforward isometric contractions at the corresponding lengths. These measurements revealed no important differences in the tensions produced in the two cases; for instance, in a typical experiment in which the measurements were made at L_0-1 mm the following results were obtained:

Tension redeveloped after a release from L_0+1 mm: 200 g wt.

Tension developed in an isometric contraction: 210 g wt.

Tension redeveloped after a release from L_0+1 mm: 210 g wt.

Although quick releases were always made over the same range of muscle lengths (L_0+1 to L_0-1 mm), the amount of tension redevelopment at L_0-1 mm varied from 70 to 95% in different muscles. The reason for the large range of variation is that in a muscle which only has a skeletal attachment at one end, referring all the length measurements to the body length (L_0) of the muscle does not introduce as much standardization into the measurements as it does in the case of muscles, such as the frog sartorius, which have skeletal attachments at both ends. The body length varies so much in muscles taken from animals of the same size that the regular use of releases from L_0+1mm to L_0-1 mm is obviously not a guarantee that one is necessarily working over the same part of the tension–length curve in different muscles. By examining the tension–length relationship (see Abbott & Lowy, 1958*b*), it can be seen how the tension redevelopment could vary considerably according to the initial length from which the releases are made.

Measurements have also been made of the tension redevelopment after releases at different speeds, but Abbott & Lowy's (1958*a*) finding that the tension redevelopment depends on the release speed has not been confirmed. Figure 4 shows some of the results of an experiment in which releases of 2 and 3 mm were made at velocities from 0·5 to 50 mm/sec. When a series of 2 mm releases were made at 50, 5, 0·5, 5 and 50 mm/sec, the corresponding amounts of tension redevelopment were 92·2, 93·4, 95·0, 92·3 and 91·8%, respectively. This series was highly satisfactory in that the amount of fatigue shown by the muscle was almost negligible; the tension immediately before the release in the last of the series was only 1% smaller than that in the first. In a further series of 3 mm releases at 50, 5 and 0·5 mm/sec the tension redevelopments

were 77·2, 80·0 and 75·3%, respectively. Thus, contrary to the findings of Abbott and Lowy, these results do not suggest that the amount of tension redevelopment is in any way dependent on the speed of the release.

Tonic response

During stimulation. When the ABRM is released during stimulation in a tonic response (i.e. while it is immersed in ACh), its behaviour is similar to that seen when releases are made during a phasic response. Figure 3*b* shows

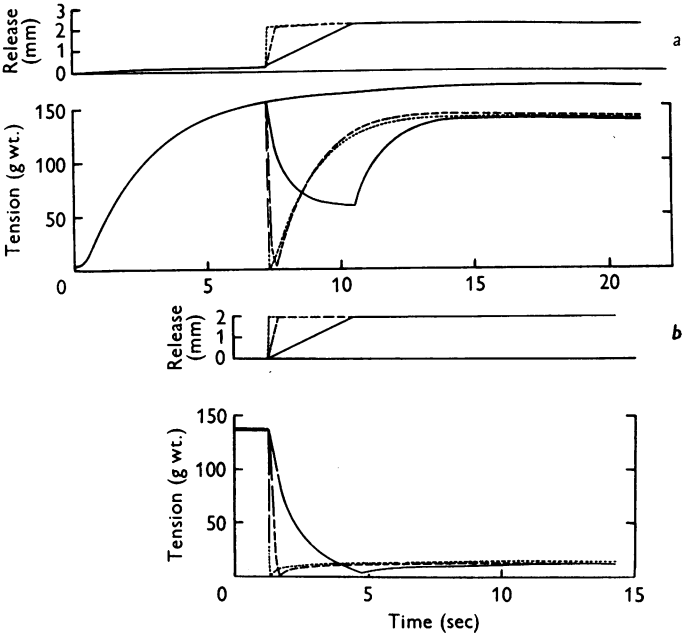

Fig. 4. Redevelopment of tension after releases at different speeds. ABRM: L_0 = 28 mm weight = 52 mg; temp. 19° C. Records: tracings from simultaneous recordings of the length and tension in the muscle made on quarter-plate paper. Releases: from L_0+1 mm to L_0-1 mm at 0·5 mm/sec (solid lines), 5 mm/sec (interrupted lines), and 50 mm/sec (dotted lines). (*a*) Releases made during phasic responses (7·3 sec after the onset of stimulation). (*b*) Releases made after stimulation in a tonic response (induced by previous treatment with ACh).

the tension changes that occurred when a muscle was released from L_0+1 mm to L_0-1 mm at 50 mm/sec, 50 sec after it was immersed in ACh solution; the tension fell to zero and then redeveloped (30 sec later) to 93·5% of the value immediately before the release. In this muscle, the redevelopment of tension in ACh solution was actually greater than that recorded when a quick release was made during an isometric tetanus.

After stimulation. The ABRM behaves quite differently when it is released after stimulation in a tonic response (i.e. after the ACh has been removed).

Release 3 (Fig. 3*c*) was identical in every other respect with the one made during stimulation in Fig. 3*b*, but on this occasion the tension only redeveloped to 20·3% of the value recorded immediately before the release. When the muscle length was restored to L_0+1 mm, 10 sec after release, the tension first overshot and then returned along a roughly exponential time course to a final steady level that was only slightly less than the value before the release. This sequence of length changes (release and stretch) was repeated on two further occasions a few minutes later (Releases 4 and 5) and the corresponding tension changes are given in Fig. 3 (caption). This inert behaviour was seen *after* stimulation in tonic responses induced by both ACh and d.c. stimulation. The amount of tension redevelopment varied from 10 to 25% in different muscles and, as Fig. 4*b* shows, it was independent of the release speed over the range 0·5–50 mm/sec.

The figures in the last two rows of the table given beneath Fig. 3 show that although the ability of the muscle to redevelop tension is much less *after* stimulation than it is *during* stimulation in a tonic response, the muscle can still maintain tension if it is held at constant length. In fact, under these conditions, the muscle exhibits the inert behaviour of a damped elastic body. Accordingly, its load–extension or tension–length curves can be determined by making releases of various sizes and measuring the corresponding tension changes. Releases could be made from the same initial length and tension by making use of the remarkable property of the tonically contracted muscle, that after a quick release, the tension can be restored to its initial value by simply re-stretching the muscle.

In the actual experiment, the tension and length of the muscle were displayed on the two beams of a Cossor 1049 oscilloscope. After the muscle had been treated with ACh (10 μg/ml.) and thoroughly washed, the positions of the length and tension beams were adjusted so that they exactly coincided (Fig. 5). When the muscle was re-stretched after each release, the tension overshot and then fell slowly as stress-relaxation occurred; as soon as it had fallen to its initial level (i.e. when the positions of the beams again coincided), the next release was made. In Fig. 5 the tension and length changes that occurred in the muscle when it was released by amounts varying from 0·2 to 4·0 mm at 43 mm/sec are shown as a continuous recording.

By measuring the tension immediately after the release in each record and plotting this against the corresponding amount of release, it is possible to construct the load–extension curve of the series elastic elements (see Fig. 6). The curves obtained by analysing five sets of records like that shown in Fig. 5 all fell within the envelope enclosed by curves a_1 and a_2 in Fig. 6. The load–extension curve obtained immediately afterwards by plotting the tension in the muscle against its length during a release at 43 mm/sec (controlled-release method) is shown by curve a_3. The agreement between this curve and the

others shows that the method illustrated by Fig. 6 gives a reasonably accurate estimate of the series elasticity of the ABRM. The other curves shown in this figure are the load–extension curves that were obtained by plotting the tension 10 sec after the release against the corresponding amounts of release; in fact the tension was still rising 10 sec after the release, but it was only about 20% less than the final steady value that was reached 1–2 min later. Five sets of records were analysed and the curves obtained all fell within the envelope enclosed by curves b_1 and b_2. These represent the tension-length relationship of the ABRM *after* stimulation in a tonic response. A further demonstration

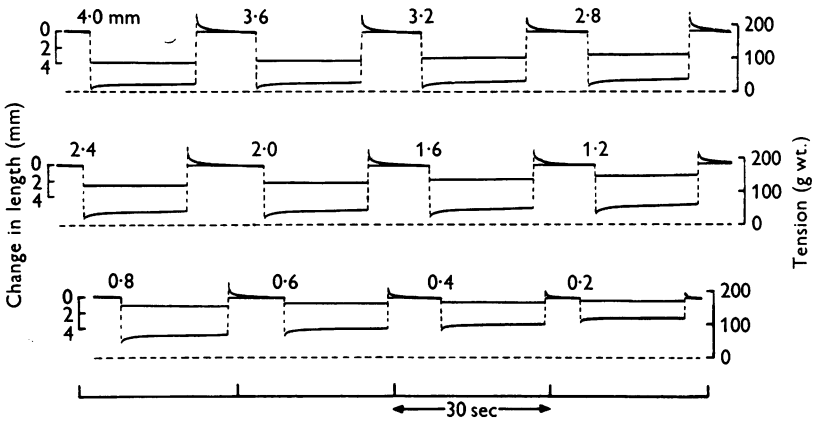

Fig. 5. Tension changes produced by releasing the ABRM by various amounts after stimulation in a tonic response. ABRM: L_0 = 27 mm, weight = 52 mg; temp. 20° C. 35 mm records: one trace shows the changes of length in the muscle, the other shows the corresponding changes of tension; the records have been retouched where they were too faint to reproduce satisfactorily. Interrupted lines have been added to show zero tension. Releases: from $L_0 + 1$ mm at 43 mm/sec. After the muscle had been treated with ACh and then washed thoroughly, the positions of the length and tension beams were adjusted so that they exactly coincided. The muscle was then released by 4 mm (records in top left-hand corner); 20 sec later it was re-stretched to $L_0 + 1$ mm. This caused the tension to overshoot, but it then fell slowly to its initial level (as shown by the coincidence of the two traces). The same sequence of changes of length was then repeated for amounts of release down to 0·2 mm.

of the inert state of the muscle under these conditions is provided by the fact that even though this experiment involved about 70 releases and stretches and took about an hour to complete, the tension at $L_0 + 1$ mm only fell by 12%.

Shortening in isotonic quick releases

Phasic response

Figure 7*a* shows the tension and length changes, respectively, in an isometric tetanic contraction and in an after-loaded isotonic contraction against a load of 30 g wt. These two types of contraction are combined when the muscle is suddenly allowed to shorten against a constant load during an isometric

contraction; Fig. 7*b* shows a typical sequence of length and tension changes as the conditions change from isometric to isotonic. When the tension is reduced, the muscle length changes rapidly at first as the series elastic elements shorten, and then more slowly as the muscle shortens actively at a velocity appropriate to the value of the load. This is the familiar picture that is seen in isotonic releases of the frog sartorius muscle (see Jewell & Wilkie, 1958).

In each experiment releases were only made against one load, and this was always arbitrarily chosen to be about 25% of the tension developed in an

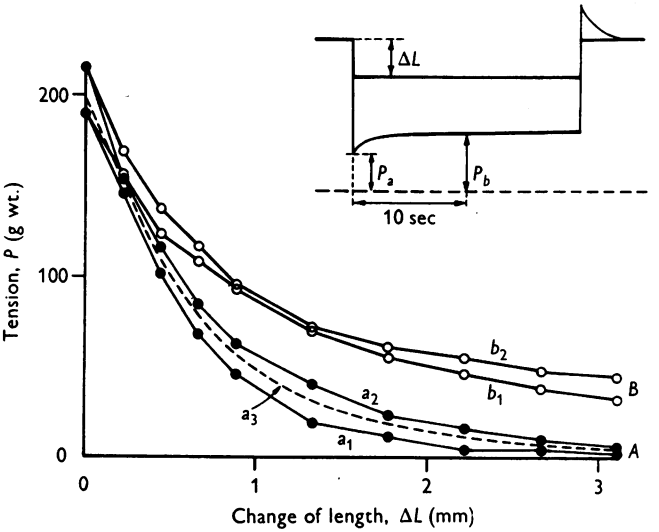

Fig. 6. Load-extension curves of the ABRM after stimulation in a tonic response. ABRM: same muscle as in Fig. 5. The measurements shown in the inset diagram were made on five sets of records like those shown in Fig. 5. (*A*) Load extension curve of the undamped series elastic elements; the points obtained by plotting the tensions immediately after the releases (P_a) against the corresponding amounts of release (ΔL) all fell in the envelope enclosed by curves a_1 and a_2. Curve a_3 was obtained by means of the controlled-release method (release speed 43 mm/sec). (*B*) Load-extension curve (tension–length relationship) of the whole muscle; the points obtained by plotting the tensions 10 sec after the releases (P_b) against the corresponding amounts of release fell in the envelope enclosed by curves b_1 and b_2.

isometric tetanus. In the experiment illustrated by Fig. 7*b*, the active shortening (total shortening minus elastic shortening) 15 sec after the muscle had been released against a load of 30 g wt. (Release 1) was 3·7 mm, but in after-loaded isotonic contractions before and after (latter is not shown), the corresponding shortenings were 4·7 and 4·4 mm, respectively. Similarly, in another experiment, the active shortenings produced in release and after-loaded isotonic contractions were 5·8 and 7·0 mm respectively. These results show that the ability of the ABRM to shorten actively in a tetanic response is diminished if the shortening is preceded by an isometric phase of contraction. This finding will be discussed later.

Tonic response

During stimulation. The few measurements that have been made of the shortening produced when the ABRM is released under isotonic conditions while it is immersed in acetylcholine solution have all shown that the ability of the muscle to shorten actively under these conditions is at least as great as it is during a phasic response. For instance, in the second experiment mentioned in the preceding section, the maximum shortening of 5·8 mm that was produced when the muscle was released during a phasic response was reached 23 sec

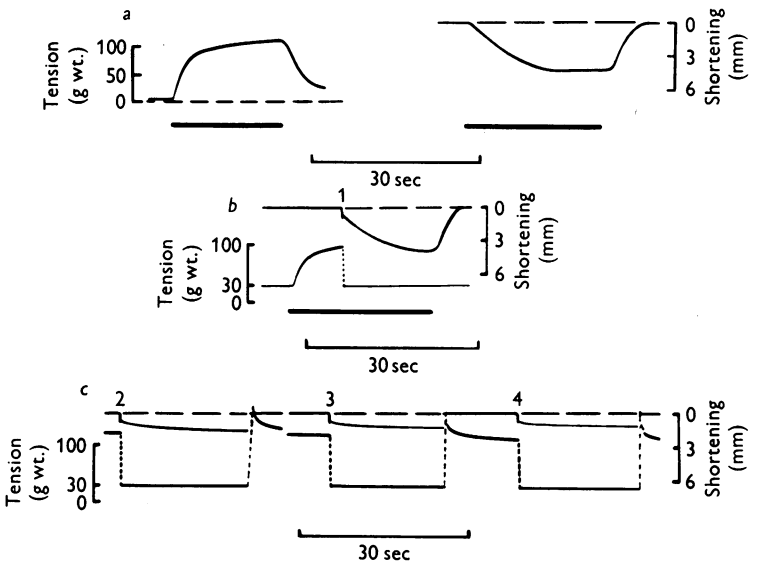

Fig. 7. Shortening in isotonic releases made during phasic and tonic responses. ABRM: L_0 = 28 mm, weight = 43 mg; temp. 22° C. 35 mm records: upper trace shows the length changes in the muscle, and lower trace shows the tension changes; the records have been retouched where they were too faint to reproduce satisfactorily. Interrupted lines have been added to show the base lines for the length and tension records, and to show when the muscle was being stimulated. Releases: from $L_0 + 1$ mm against a load of 30 g wt. (*a*) Phasic response: the first record shows an isometric tetanus; the second record shows an after-loaded isotonic contraction against a load of 30 g wt. (*b*) Isotonic release during a phasic response, 10 sec after the onset of stimulation (Release 1). (*c*) Isotonic releases after stimulation in a tonic response; these records show the effect of releasing the muscle and then re-stretching it to its initial length (Releases 2, 3 and 4) after it had been treated with ACh and then thoroughly washed.

The following results were obtained by analysing the records:

Release	Tension before release (g wt.)	Active shortening during release (mm)
1	104	3·70
2	129	1·00
3	126	0·70
4	120	0·67

after the release; when a comparable release was made with the muscle immersed in ACh solution, the shortening 23 sec after the release was 5·7 mm, but in this case the muscle continued to shorten until, after 1 min, the total change of length was 7·3 mm.

After stimulation. The isotonic releases that are shown in Fig. 7*c* were made after the muscle had been treated with ACh and then thoroughly washed; after each release, the muscle was restretched to its original length and the tension was restored to roughly its original value. The results given in the last three rows of the table beneath Fig. 7 were obtained by measuring the tension and length changes during and after these releases. They show that the ability of the muscle to shorten actively is much less *after* stimulation than it is *during* stimulation, and they also provide further evidence that the ABRM behaves like a damped elastic body after stimulation in a tonic response.

DISCUSSION

Phasic response

Although the work of Fletcher (1937*a*, *b*, *c*) and Winton (1937) had shown that the properties of the ABRM during a phasic response are similar to those of vertebrate striated muscle under comparable conditions of excitation, the full extent of the similarity was not realized until Abbott & Lowy (1958*a*, *b*) investigated the thermal and mechanical properties of the ABRM during phasic responses. The release experiments described in this paper provide further evidence in support of the view that the contractile mechanisms of the two types of muscle are fundamentally the same.

The character of the isotonic quick-release records shown in Fig. 7 reveals the presence of largely undamped elastic elements in series with a contractile component, and the measurements of the tension changes during quick releases of different magnitudes allowed a determination to be made of the load–extension curve of the elastic elements (Fig. 6). The procedure used in this method of measuring the series elasticity is the exact converse of that used in the isotonic quick-release method (Wilkie, 1956), in which the length changes are measured after the tension has been reduced by known amounts. Strictly speaking it is only a variation of the controlled-release method (Hill, 1950; Jewell & Wilkie, 1958), which allows the same measurements to be made during a single constant-velocity release. Measurements of the series elasticity have been made on a number of muscles and the results show that a length change of 3–4 % of the muscle length is required to reduce the stress in the muscle from 8 kg wt./cm^2 to zero. This means that the series elastic component of the ABRM is probably less compliant than that of the frog sartorius, in which a length change of 2–3 % is required to reduce the stress from 2 kg wt./cm^2 to zero (Jewell & Wilkie, 1958).

The amount of tension redevelopment after quick releases was shown to be

consistent with the known shape of the tension–length relationship of the muscle (Abbott & Lowy, 1958*b*); this means that the ability of the ABRM to develop tension is not impaired by a sudden change in length. The present experiments have not confirmed Abbott & Lowy's (1958*a*) finding that 'the slower the speed of release of active molluscan muscle, the smaller the subsequent redevelopment of tension'. The results shown in Fig. 4 demonstrate that the amount of tension redevelopment is independent of the speed of release over a wide range of velocities (0·5–50 mm/sec).

The records obtained from the ABRM (see Figs. 3*a* and 4*a*) are like those obtained from other muscles, in that the redevelopment of tension after a quick release is always more rapid than the initial development of tension in an isometric tetanus. Goodall (1957) demonstrated this in the retractor penis muscle of the tortoise, and Jewell & Wilkie (1958) have shown that it also occurs, but to a lesser degree, in the frog sartorius muscle (see also Hill, 1953). The explanation is almost certainly the same in the three cases—the active state is not fully developed immediately after the first shock; thus the muscle is not fully active during the initial rise of tension, but it is during the redevelopment after a release.

Measurements made under isotonic conditions have shown that the active shortening produced by the ABRM during an isotonic release is invariably less than that produced during an after-loaded isotonic contraction against the same load. This phenomenon has been observed in other muscles on several occasions: by Buchtal (1942) and Buchtal, Kaiser & Rosenfalck (1951) in isolated frog muscle fibres; by X. M. Aubert and Nuyts (unpublished work, quoted by Abbott & Aubert, 1952) in the frog sartorius; and by Abbott & Aubert (1952) in the dogfish jaw muscle: but this form of 'inactivation' is not at all clearly understood. To explain this finding and the fact that in the dogfish jaw muscle the amount of tension redevelopment after a quick release is dependent on the release speed, Abbott & Aubert (1952) suggested that the contractile links become firmly bonded during the isometric phase of the contraction, so that the muscle cannot shorten freely when the conditions suddenly become isotonic. It is worth remembering that the force–velocity curves obtained from the frog sartorius during after-loaded and release isotonic contractions show remarkable agreement (Jewell & Wilkie, 1958, fig. 5), so it is not the initial velocity of shortening, but the total amount of shortening, that is affected by this form of 'inactivation'. This observation makes it all the more difficult to visualize the nature of the 'irreversible' changes that are produced in the muscle during the isometric phase of the contraction. The reason why the ability of the muscle to redevelop tension is not similarly affected is presumably that the amount of shortening required of the contractile component is comparatively small under isometric conditions, so that the effects of 'inactivation' do not become apparent.

Hoyle & Lowy (1956) believe that the phasic response is accompanied by a transient increase in the rate of firing of spontaneously active elements in the muscle, and it has been argued that 'repetitive shocks produce "spontaneous" activity, not a tetanic response as in frog sartorius ' (Lowy & Millman, 1959). However, other workers (Fletcher, 1937*b*; Prosser, Curtis & Travis, 1951; Schmant & Sleator, 1956) have all found that repetitive stimuli at frequencies below about 10/sec produce synchronous action potentials which bear a strictly 1:1 relationship to the number of stimuli. The fact that it is possible to obtain a series of phasic responses in which the isometric myograms are identical in every detail also makes it seem unlikely that the contraction produced under these conditions is the direct result of asynchronous increases in the discharge rates of spontaneously active elements in the muscle.

Tonic response

The results obtained by making release experiments during the tonic response produced by treating the ABRM with acetylcholine have shown that the mechanical properties of the muscle are different *during* and *after* stimulation. Although the properties of the muscle were not investigated in detail during immersion in acetylcholine solution, the results suggest that they are the same as those found during a phasic response. On the other hand, the mechanical properties were shown to be quite different when the acetylcholine was removed, and the muscle was found to show the inert behaviour of a damped elastic body. Accordingly, measurements were made of the load–extension curves of the undamped part (the series elastic elements) and of the whole muscle (Fig. 6). These two curves have been replotted on a different abscissal scale in Fig. 8 (curves *A* and *B*) and a curve has been added to show the estimated tension–length relationship of the muscle during stimulation in phasic and tonic responses (curve *C*). Lines have been drawn on these graphs to show how the amounts of tension redevelopment (Fig. 8*a*) and the amounts of shortening (Fig. 8*b*) differ when releases are made during and after stimulation in a tonic response.

When stimulation ends in a tonic response (i.e. when the acetylcholine is removed) the tension–length relationship of the muscle changes from the form shown by curve *C* in Fig. 8 to that shown by curve *B*, and this seems to correspond with the variations in 'viscosity' and 'plasticity' described by Winton (1937) and Johnson (1954), respectively. In the present experiments, it was not possible to determine the time course with which this change takes place, because the muscle had to be washed thoroughly after the acetylcholine was removed; all one can say is that the change is complete within a few minutes. In this respect, direct-current stimulation is much more satisfactory in that releases can be made immediately after stimulation ceases; measurements made in this way show that the change takes place within a few seconds

of the end of stimulation (W. H. Johnson, personal communication). Lowy & Millman (1959) have shown that the active state produced by a single shock also decays to zero with this sort of time course.

One can only speculate on the nature of the mechanism that is responsible for supporting the tension after stimulation has ended in a tonic response. The results of mechanical measurements alone do not allow one to say whether

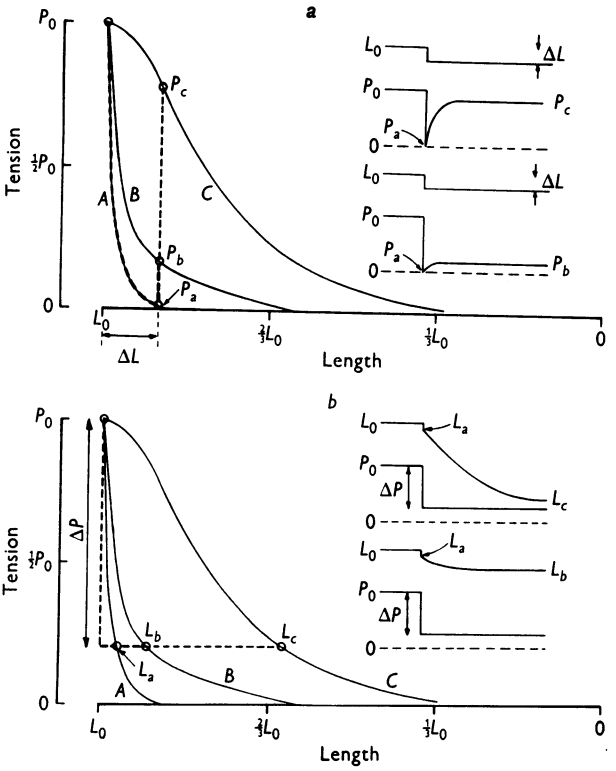

Fig. 8. Diagrams illustrating the behaviour of the ABRM when it is released during and after stimulation in a tonic response. The means of the curves shown in Fig. 6 have been replotted on a different abscissal scale; *A* is the load–extension curve of the series elastic elements, and *B* is the tension–length relationship of the muscle *after* stimulation in a tonic response. Curve *C* shows the estimated shape of the tension–length relationship of the muscle *during* stimulation in phasic and tonic responses. (*a*) Redevelopment of tension after quick releases. When the muscle is released by an amount, ΔL, during stimulation, the tension falls from P_0 to P_a as the series elastic elements shorten, and it then redevelops to P_c. When the release is made after stimulation, the elastic elements shorten as before and the tension falls to P_a, but it only redevelops to P_b. (*b*) Shortening during isotonic releases. When the tension is suddenly reduced by an amount, ΔP, and the muscle is allowed to shorten freely against an isotonic load, the series elastic elements first shorten rapidly to L_a, and the muscle length then changes slowly to L_c as the contractile elements shorten. When the release is made after stimulation, the elastic shortening to L_a is the same, but the subsequent shortening produced by the contractile elements only brings the muscle length to L_b.

the difference in the muscle's ability to shorten actively during and after stimulation is simply quantitative, or whether the origin of the shortening is quite different under the two conditions. The mechanical properties of the ABRM after stimulation strongly suggests that the muscle is in a state of 'reversible rigor', but measurements of the heat production of the muscle under these conditions (J. V. Howarth & B. R. Jewell, unpublished) show that it is above the resting level. It therefore seems unlikely that this muscle could ever support tension without some additional expenditure of energy. From the similarities between the ABRM and vertebrate muscle one is tempted to interpret the experimental findings in terms of a contractile mechanism which is based on actin and myosin and which contracts by splitting adenosine triphosphate, but the results of recent investigations on the micro-structure and protein composition of other lamellibranch muscles (see Hanson & Lowy, 1957) suggest that the mechanism is probably a good deal more complicated than this; in particular, it seems likely that the protein, tropomysin *A*, which has negligible adenosine triphosphatase activity, but which is present in abundance in these muscles, is in some way responsible for their ability to maintain large tensions economically for long periods (Rüegg, 1958). It is unfortunate that most of these investigations have been made on the adductor muscles, which are totally unsuitable for making isolated intact preparations for thermal and mechanical studies.

The nature of the phasic and tonic responses

In this section, two separate issues must be considered: first, the relationship between phasic and tonic responses; and secondly, the nature of the tonic response.

In the preceding section, the tonic response was discussed and it was concluded that the properties of the ABRM are strikingly different during and after stimulation. Furthermore, the rate of heat production of the muscle seems to be much greater during stimulation than it is after stimulation (J. V. Howarth & B. R. Jewell, unpublished). These observations strongly suggest that two distinct mechanical states are possible in the ABRM—an 'active' state, associated with a high rate of energy expenditure, in which the muscle can shorten actively; and a 'fused' state, associated with a low rate of energy expenditure, in which tension can be maintained if the muscle is held at constant length, but in which little active shortening is possible. The 'active' state present in the muscle during stimulation gives way to the 'fused' state within a few seconds when stimulation ends: this then decays with an extremely slow time course, and the energy expenditure of the muscle returns to its resting level.

The relationship between the phasic and tonic responses. If it is accepted that these two mechanical states can exist in the ABRM, then a reasonable explana-

tion can be given of the well-known fact that the effects produced by different types of electrical and pharmacological stimuli appear to depend on the nature of the stimulus and on the initial state of the muscle (see Winton, 1937; Twarog, 1954). The hypothesis that will be put forward is an elaboration of an idea suggested by van Nieuwenhoven (1947), and it is based on his finding that the ABRM is supplied with both excitatory and inhibitory nerves, which reach the muscle fibres via intricate, intramuscular nerve plexuses (Bowden & Lowy, 1955). It is postulated that:

1. Different types of stimuli act on either the excitatory or the inhibitory systems, or on both.

2. Stimuli that activate the excitatory system (e.g. acetylcholine, direct current) produce a *tonic* response; that is, a response in which the 'active' state disappears rapidly when stimulation ends, leaving the muscle in the 'fused' state.

3. Stimuli that activate the inhibitory system (e.g. 5-hydroxytryptamine) abolish the 'fused' state, and they can therefore be used to terminate a tonic response.

4. The effects produced by stimuli that activate both the excitatory and the inhibitory systems (e.g. repetitive shocks of various types) depend on the initial state of the muscle. (i) If it is in the relaxed state, then they produce a *phasic* response—the activity in the excitatory system produces an 'active' state, but the 'fused' state that would normally follow is quickly abolished because there has been simultaneous stimulation of the inhibitory system; the result is that the tension returns to its initial resting value within about 30 sec of the last shock. (ii) If the muscle is initially in the 'fused' state, the effects produced by the stimuli will depend on the tension; if it has already fallen to a low value, there will be a contraction followed by rapid relaxation, as in a phasic response; but if the tension is still high, there will simply be rapid relaxation without any preceding phase of contraction. (iii) If the muscle is initially in the 'active' state, then the effect produced by the stimuli will depend on the number of muscle fibres active; if they are all active, the stimuli will have no apparent effect, but if only a few are active, then a phasic response will be superimposed on the existing contraction in the muscle.

In stating this hypothesis stimuli have been said to act on the inhibitory and excitatory 'systems'; this terminology has been used because stimuli that produce the same effect do not necessarily act at the same site in the muscle. For instance, it seems probable that (i) repetitive shocks act by setting up synchronous action potentials in the muscle fibres (Fletcher, 1937*b*; Schmant & Sleator, 1956), and by stimulating the intramuscular branches of the inhibitory (and possibly the excitatory) nerve supply (Hoyle & Lowy, 1956), (ii) direct current acts directly on the contractile mechanism (Fletcher, 1937*c*; Cambridge & Holgate, 1959), (iii) acetylcholine acts by depolarizing the muscle

fibre membranes (Twarog, 1954), (iv) 5-hydroxytryptamine acts directly on the contractile mechanism (Twarog, 1954).

The nature of the tonic response. When the ABRM is in the 'fused' state, its mechanical properties strongly suggest that a passive mechanism is responsible for supporting the tension, but it has been shown that the rate of heat production of the muscle is above the resting level. It therefore seems probable that the maintenance of a 'fused' state requires some increase in the energy expenditure of the muscle, and it follows that the 'catch' cannot be entirely passive, as predicted by the catch-mechanism hypothesis. However, it would be foolish to reject the entire hypothesis simply on these grounds, for the similarities between the properties of the 'fused' muscle and those of a damped elastic body are very striking, and it seems likely that the maintenance of tension under these conditions does involve a 'catch', even if it is an imperfect one in some respects.

One of its imperfections seems to be that it slips, and the result is that the tension gradually decays. To explain the fact that the tension can be maintained at a steady level for long periods *in vivo*, it is therefore necessary to postulate that there is occasional re-excitation of the muscle; in other words, that the contraction is tetanic in nature. In fact, it appears that two distinct types of tetanic contraction are possible in the ABRM. One of them resembles the tetanus seen in the frog sartorius in that it is associated with a high rate of energy expenditure, because an 'active' state is continuously maintained in the muscle; this is the type of tetanic contraction that can be produced by applying repetitive shocks (at, say, 2/sec) to the ABRM. However, it seems reasonable to suppose that the tonic response produced by the muscle *in vivo* is based on a different type of tetanic contraction, in which tension is maintained much more economically, because the muscle is only intermittently in the 'active' state. In the intervening periods, when it is in the 'fused' state, the tension is supported by the 'catch', which only requires a low rate of energy expenditure to keep it functioning. Because the 'catch' slips, it has to be reset periodically (say every 30 min) by further bursts of excitatory activity, which temporarily create an 'active' state in the muscle. The net effect is that the tension can be maintained for long periods with a very low over-all expenditure of energy.

Although no one can reasonably deny that the tonic response produced by the ABRM *in vivo* requires occasional re-excitation of the muscle, there is still some doubt about the nature of the tonic response that can be produced by stimulating the isolated muscle. Hoyle & Lowy (1956) reported that the tonic responses produced by acetylcholine and by direct-current stimulation were accompanied by asynchronous bursts of action potentials, which persisted long after the stimulus was withdrawn. However, Fletcher (1937*b*), whose recording techniques were at least as good as those used by the later workers,

was unable to detect any activity of this sort (except occasionally in damaged preparations) during or after direct-current stimulation, and recently Johnson (1958) reported that he has also been unable to detect any electrical activity in the muscle during the slow relaxation that followed cathodal stimulation with direct current in a Taylor triangular electrode. Thus it is by no means universally agreed that re-excitation of the muscle does occur when tonic contractions are produced *in vitro*.

The tetanus hypothesis of Hoyle & Lowy (1956) is based on the assumption that there are increases in the rates of firing of the spontaneously active elements during both phasic and tonic responses, the increase being transient in the phasic response, and prolonged in the tonic response, so that it greatly outlasts stimulation. They believe that these two types of response are tetanic contractions that differ in duration, and it is implied that they are related in the same way that long and short tetani are related in the case of the vertebrate skeletal muscle. If the muscle continues to be re-excited after stimulation has ended, then there is no reason to expect a sudden change in the mechanical state of the muscle at any time during the tonic response. The fact that the 'active' state does give way fairly abruptly to a 'fused' state when stimulation ends therefore makes it seem highly unlikely that there is any further re-excitation of the muscle. The slow fall in tension that occurs in the tonic response produced by stimulating the isolated muscle can adequately be accounted for by the intrinsically slow rate of decay of the 'fused' state, and there is certainly no need to postulate that re-excitation of the muscle occurs under these conditions.

SUMMARY

1. The mechanical properties of the anterior byssal retractor muscle (ABRM) of *Mytilus* have been investigated during phasic and tonic responses by means of release experiments, in which measurements were made of the muscle's ability to shorten and to redevelop tension when it is released during isometric contractions.

2. Phasic responses were produced by stimulating the muscle on a multi-electrode assembly, with alternating condenser shocks at 10/sec. Tonic responses were produced by treating it with acetylcholine.

3. When releases were made during phasic responses, the mechanical properties of the muscle were found to be qualitatively similar to those of active vertebrate skeletal muscle. After quick releases the redevelopment of tension was found to be consistent with the shape of the tension–length relationship, and independent of the speed of release over the range 0·5–50 mm/sec. In isotonic releases the total active shortening was always less than that recorded in after-loaded contractions against the same load.

4. When releases were made during tonic responses, the mechanical

properties of the muscle were found to differ during and after stimulation. *During* stimulation the muscle's ability to shorten and to redevelop tension was at least as great as it was during phasic responses; but *after* stimulation the amount of active shortening and the redevelopment of tension were only about 25% of the corresponding values found during stimulation.

5. The behaviour of the muscle after stimulation resembled that of a damped elastic body, and determinations were made of the load–extension curves of the undamped series elastic elements, and of the whole muscle.

6. It was suggested (i) that two mechanical states are possible in the ABRM —an 'active' state, associated with a high energy expenditure, and a 'fused' state, associated with a low energy expenditure, (ii) that the 'active' state present during stimulation in a tonic response disappears when stimulation ends, leaving the muscle in a 'fused' state, which decays extremely slowly, and (iii) that this provides the muscle with an economical means for supporting tensions for long periods, both *in vitro* and *in vivo*.

7. The varied effects that are produced by different types of stimuli were explained by supposing that the ABRM contains both excitatory and inhibitory 'systems', which may be activated independently or simultaneously according to the nature of the stimulus.

8. The nature of the tonic response was discussed, and it was shown that the behaviour of the ABRM shows some of the features predicted by both the 'catch-mechanism' and the 'tetanus' hypotheses.

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Note added in proof. In a recent paper, Lowy & Millman (1959, *Nature, Lond.*, **183**, 1730–1731) have postulated that 'passive' tension ('fused' state in my terminology) is due to tonic linkages in a paramyosin (i.e. tropomyosin A) system. Their hypothesis is also similar to the one advanced in this paper in that they suggest that the rate at which these tonic linkages disappear can be increased by inhibitory stimulation.