The Pattern of Activation in the Sartorius Muscle of the Frog

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ABSTRACT The development of isometric twitch tension has been compared with the redevelopment of isometric tension in the fully active frog sartorius muscle following release. At 0°C the rate of rise of isometric twitch tension is the same as that for the muscle in the fully active state at the same tension but not until about 40 msec. after the stimulus and then only for a few milliseconds. The rates of rise of tension in the twitch and in the redevelopment of tension in the fully active muscle appear to be nearly the same at low tensions. Substitution of nitrate for chloride in the Ringer's solution bathing the muscle retards the development of tension during the early part of the contraction phase of the twitch and the effect reaches a maximum within 3 minutes after changing the solutions. These observations have been discussed in connection with some possible patterns of activation and the hypothesis has been advanced that the rate of activation of a sarcomere is determined mainly by the rate at which the transverse component of the link between excitation and contraction is propagated inwards from the periphery to the center of the fiber. This hypothesis has been discussed in relation to others concerning the nature of excitation-contraction coupling.

INTRODUCTION

Excitation of the muscle fiber membrane initiates a sequence of events which leads to the activation of the contractile material within the fiber. This process has been referred to as excitation-contraction coupling and the subject has been reviewed by Sandow (1952), Botts (1957), and Gelfan (1958). Several hypotheses have been advanced concerning the nature of this coupling mechanism (Sandow, 1952; Bay *et al.*, 1953; Fleckenstein, 1955; Frank, 1958; Csapo, 1959; Huxley, 1959) but no data on the temporal and spatial aspects of this process have been recorded. In the present work an attempt has been made to interpret the mechanical behavior of the muscle in this connection.

The approach to this problem has been first, to determine the degree of maximum activation in the twitch and the time after the stimulus at which this occurs; second, to interpret the mechanical behavior of the muscle during the period of activation in terms of a possible pattern of distribution of active contractile elements; third, to relate this pattern of activation to the mode of excitation-contraction coupling.

It is known from the work of A. V. Hill and others (Wilkie, 1956) that active striated muscle behaves essentially as a two component mechanical system composed of an undamped elastic element in series with the contracting substance. The tension:time curve in an isometric tetanus is determined partly by the relationship between the force and the speed of shortening of the contractile component and also by the load:extension properties of the series elastic elements (Hill, 1938, 1949 *b*).

In 1924, Gasser and Hill proposed a "fundamental mechanical response" in an attempt to describe the twitch tension:time curve which would be seen if the series elastic element were not present and if the contractile component were unable to shorten. In recent years this has come to be known as the "active state curve." Hill (1938, 1949 *b*) has shown that the fully active contractile component is defined by the three constants a, b, and P_o in the following equation which relates speed of shortening and load:

$$(P + a)V = b(P_o - P)$$

P and V are the load and speed of shortening respectively; P_o is the maximum isometric tetanic tension; a has the units of force and b has the units of velocity. The intensity of the active state is defined by Hill's equation as the load which the contractile component could bear if it were neither lengthening nor shortening.

In connection with the question concerning the degree of maximum activation following a single stimulus, Hill (1949 *b*) has shown that if the series elastic element is extended, by applying an appropriate stretch, the muscle can then bear a load nearly equal to the tetanic tension for a short time after the stimulus. In addition, the characteristic equation was found to be obeyed early in the twitch with the same constants as were found for tetanic contractions (Hill, 1949 *a*). These observations have led Hill to conclude that the contractile component is fully activated for a short time during the early part of the contraction phase of the twitch.

Little is known about the time course of the development of the active state following a single stimulus at 0°C. There can be little doubt that the fully active state is developed very early in the twitch since shortly after the onset of contraction the muscle can bear a load equal to the maximum isometric tetanic tension (Hill, 1949 *b*). Hill (1950) has also shown that striated

muscle develops a markedly increased resistance to stretch shortly after stimulation and he has suggested that this increased rigidity is a property of the contractile component when in the active state. In frog sartorius muscle at 0°C this increase in rigidity begins within a few milliseconds after a stimulus (Hill, 1951 *b*) and reaches a maximum within about 40 msec. (Hill, 1949 *b*). Other interpretations have also been based upon indirect evidence and there is considerable difference of opinion as to the exact time after the stimulus at which the muscle becomes fully active. Hill (1951 *a*) and Abbott and Ritchie (1951) have suggested that there is a fairly abrupt transition from rest to full activity within 20 to 25 msec. after the stimulus. Macpherson and Wilkie (1954) believed that there is a "plateau" of full activity lasting from the onset of contraction until about 45 msec. after the stimulus. Ritchie and Wilkie (1958) have suggested that the active state takes some time to reach its maximum and Jewell and Wilkie (1958) have presented evidence indicating that in tetanic contractions the muscle is not fully activated until about 60 msec. after the onset of contraction.

In the present work the development of isometric twitch tension has been compared with the development of isometric tension during a clonic contraction, and with the redevelopment of isometric tension in the fully active (tetanized) muscle following release to a new length. It has been found that in isometric twitch contractions it is not until about 40 msec. after the stimulus that the muscle behaves as though it were fully activated. The location of processes which might require this time to run to completion and the spatial gradation of activity during the development of the active state will be discussed.

Two effects of nitrate ions upon the form of the isometric twitch are described in this work. In addition to the well known potentiation of maximum twitch tension (Kahn and Sandow, 1950) it has been found that the development of tension is retarded in the early part of the contraction phase of the twitch during the period of development of the active state. The potentiating effect is known to be a result of delayed decay of the active state (Ritchie, 1954) which is brought about by an action of the nitrate ions at, or near, the surface of the muscle fiber (Kahn and Sandow, 1950; Ritchie, 1954; Hill and Macpherson, 1954). Hill and Macpherson have also shown that nitrate ions in the medium bathing the muscle do not alter either the maximum isometric tetanic tension or the shortening velocity of the fully active muscle. The two effects of nitrate will be discussed in connection with the site of action of these ions and in relation to excitation-contraction coupling.

METHODS

Sartorius muscles from small frogs (*Rana pipiens*) were used in these experiments. The excised muscle was mounted vertically in a bath containing oxygenated Ringer's

solution (NaCl, 111 mM; KCl, 2 mM; $CaCl_2$, 2 mM; sodium phosphate buffer, pH 7.2, 2 mM; tubocurarine, 1/100,000 w/v). In all experiments the temperature of the bath fluid was maintained at a low level (0 to 2°C) by placing the muscle bath in a small Dewar flask containing a mixture of ice and water. The temperature of the bath was determined by means of a calibrated thermistor secured near the muscle.

Massive supramaximal stimuli were applied to the muscle between pure silver plate electrodes similar to those described by Mostofsky and Sandow (1951).

Isometric tension was recorded by connecting the distal tendon to a spring steel leaf beam by "tru-chrome" wire (0.006 inch diameter). A force applied to the leaf beam deflected the plate of a mechanoelectronic transducer (RCA 5734) and unbalanced the bridge into which the tube was incorporated (for details of the circuit, see Donaldson, 1958, p. 491). The output from the force recorder was fed to a directly coupled amplifier and displayed on one beam of an oscilloscope and photographed. In many experiments the rate of change of tension in the muscle was displayed simultaneously on the other beam.

Differentiation was achieved electrically by passing the output of the force transducer bridge through a series resistance–capacitance circuit (RC = 0.1 msec.). The source impedance was reduced by connecting the bridge to the RC circuit by means of a cathode follower (output impedance = 640 ohms) and the input impedance of the oscilloscope amplifier was kept at a minimum (about 10 K). The error in the differentiated record was calculated to be less than 0.1 msec. in the frequency range 1 to 100 CPS and was neglected. The output of the differentiating circuit was calibrated against the calculated rate of rise or fall of a sinusoidal wave.

In certain experiments the muscle was tetanized at 110 per cent of rest length then released to rest length (the natural length of the muscle in the body) in order to discharge the tension in the series elastic elements. The subsequent redevelopment of tension was recorded from zero to its full value. In this recording the rate of rise of tension was displayed on the y axis of the oscilloscope and the tension on the x axis, as has been done previously by Macpherson and Wilkie (1954).

RESULTS

Development of Isometric Tension in Twitch, Tetanic, and Clonic Contractions

Fig. 1 shows the superimposed records of the rate of rise of tension:tension curves for the development of isometric tension during a twitch and for the redevelopment of isometric tension in the fully active muscle following release; both records were obtained from the same muscle preparation. The curve for the fully active muscle was obtained by quickly releasing the muscle from 110 per cent of rest length to rest length 100 msec. after the beginning of repetitive stimulation at 33 shocks/sec. The subsequent redevelopment of isometric tension was recorded from zero tension to the maximum isometric tetanic tension during continued stimulation at 33 shocks/sec. In these experiments the rate of rise of tension was displayed on the y axis of the oscillo-

scope and tension on the x axis. The time courses of the development of tension and the rate of rise of tension during the contraction phase of the twitch were obtained from records of the type shown in Fig. 2. Quite small but rapid vibrations can occur as a result of the release and these are seen as very large excursions of the differentiated record. To obtain a useful differentiated record these vibrations were reduced to a minimum. No dampening devices were used but particular care was taken to ensure that all connections between the muscle and the force transducer were secured tightly. The large

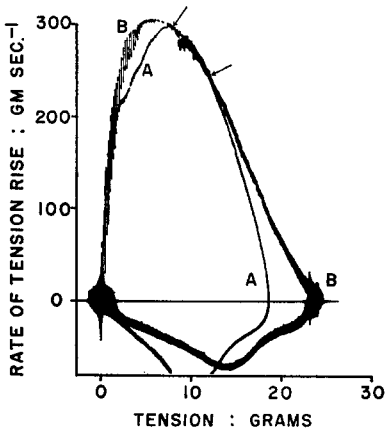

FIGURE 1. Records of the rate of change of tension:tension for the development of isometric tension in a twitch (*A*) and for the redevelopment of isometric tension in the fully active muscle (*B*) during repetitive stimulation at 33 shocks/sec. after quick release to rest length at 0.1 sec. after the beginning of stimulation. The arrows indicate the region of the twitch curve in which the instantaneous rate of rise of tension at a particular tension is the same as that for the curve for the fully active muscle. The region between the arrows is the same as that indicated by the vertical lines in Fig. 2. The undulations in the rising part of record *B* have been retouched with ink. Both records were obtained from one muscle at rest length and at 0°C. The ratio of the force constant (a) to the maximum isometric tetanic tension for this muscle was calculated from isotonic data to be equal to 0.3.

and small undulations which are superimposed on both records in Fig. 1 are due to the recording equipment.

In Fig. 1 the rate of rise of tension at low tensions appears to be nearly the same in the two records. The curve for the twitch then departs from that for the muscle in the fully active state but appears to rejoin it at tensions between about 8 to 12 gm. As seen in Fig. 2 these tensions were developed at about 42 and 55 msec. after the stimulus in the twitch of this muscle. These times could not be determined very accurately from some of the records obtained from other muscles but the two curves always appeared to coincide for 5 to 15 msec. from about 40 msec. after the twitch stimulus. At other times during

contraction, except at the onset of contraction, the rate of rise of tension during the twitch was much less than that for the fully active muscle at the same tension.

These results are qualitatively similar to those of Jewell and Wilkie in connection with their finding that for a short period of time after the onset of contraction the initial rise of tension in a tetanus is somewhat slower than the redevelopment of tension. However, their records showed that these contractions differed in this respect until 60 msec. after the onset of the initial contraction which is considerably more than the 33 msec. period (42 msec.

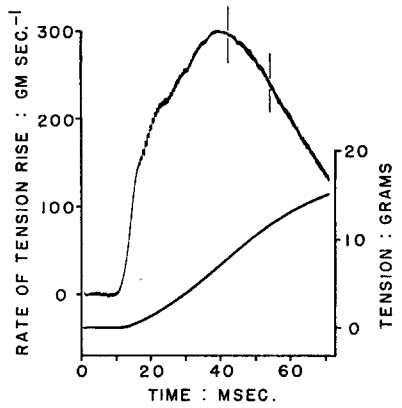

FIGURE 2. The tension:time curve (lower record) and its derivative (upper record) of the early part of the contraction phase of a twitch. These records and those in Fig. 1 were obtained from the same muscle. The regions of the curves between the small vertical lines on the differentiated record are equivalent to the region indicated by the arrows in Fig. 1. The stimulus occurred at zero on the time scale. Muscle at rest length; 0°C.

minus 9 msec. latent period) obtained for the twitch from Figs. 1 and 2 above. Differences in experimental method might easily account for this discrepancy. In this connection it should be noted that the time relations of isometric contractions are known to be dependent upon the mode of stimulation (Macpherson and Wilkie, 1954) and that in the present work the muscles were stimulated massively whereas in the experiment described by Jewell and Wilkie a multielectrode assembly was used. Massive stimulation leads to synchronous activation of all sarcomeres but with ordinary stimulation by a multielectrode assembly some parts of the muscle membrane must be excited directly by the stimulus and other parts by the propagated muscle impulse. The resulting asynchrony of sarcomere activation would be dependent not only upon the interelectrode distance but also upon the strength of the stimulus. The results of Macpherson and Wilkie (Fig. 4, 1954) provide a possible explanation for the discrepancy on that basis for they found with multielectrode stimulation that the active state did not begin to decline until 60 msec.

after a single shock when the stimulus was about twice threshold intensity whereas it declined as early as 42 msec. after much stronger shocks. Jewell and Wilkie state that some time must have been required for conduction of the muscle impulse in their experiments but that this could not have been more than 10 msec. This time in itself does not account for the discrepancy but the behavior of sarcomeres is probably different in other respects as a result of asynchronous activation. For example, more active sarcomeres would stretch those which are activated later by the propagated impulse and

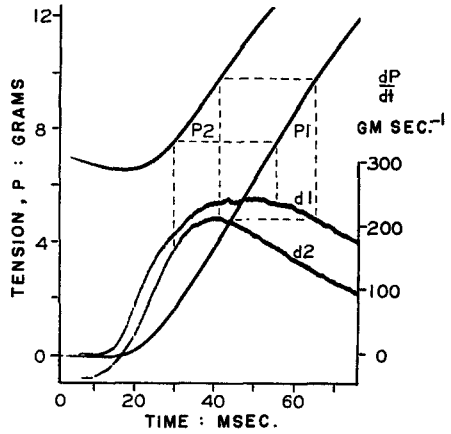

FIGURE 3. Records of tension:time curves of the early parts of the contraction phase of a twitch ($P1$) and of a clonic response to a second stimulus ($P2$) given while the muscle was relaxing about 600 msec. after the first stimulus; $d1$ and $d2$ are the derivatives of $P1$ and $P2$ respectively. The dashed lines have been drawn to indicate the rate of rise of tension at the same tension in the two responses (see text). The stimulus occurred at zero time in both cases. All four records began at zero time on the abscissa but some of the traces were not recorded properly during the first few milliseconds of the sweep because the intensity of the beam was too low. Muscle at rest length; 0°C.

this redistribution of length might retard tension development as suggested by Hill (1953). In this case the results in question would not be strictly comparable. In addition to differences in experimental method there is some artifact in the records in Figs. 1 and 2 above and in those presented by Jewell and Wilkie. More work is desirable to further reduce these artifacts but it is improbable that the records were seriously distorted. In fact the results of the present work are consistent with those from quite different experiments of Hill (1949 *b*) and Macpherson and Wilkie (1954) on two important points (discussed below), and such good agreement could not be expected if the artifact in Figs. 1 and 2 had significantly influenced the results.

The records shown in Fig. 3 are the tension:time curves, and their derivatives, of the early part of the contraction phase of a twitch and of the clonic

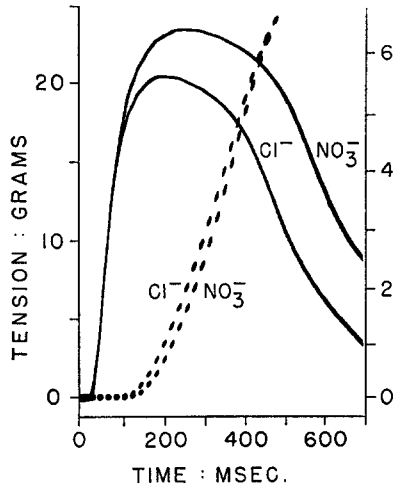

FIGURE 4. Twitch tension:time curves for a muscle in chloride-Ringer and nitrate-Ringer solutions. The early part of the twitch (modulated trace) and the greater part of the twitch were recorded simultaneously first in chloride-Ringer and then in nitrate-Ringer. The beam recording the early part of the twitch was modulated at 500 CPS and the tension scale for these records is on the right ordinate. The greater part of the twitch is shown in the unbroken records and the tension scale for these is on the left ordinate. The time scale on the abscissa is for the greater part of the twitch only. The stimulus occurred at zero on the time scale. Muscle at rest length; 1.5°C.

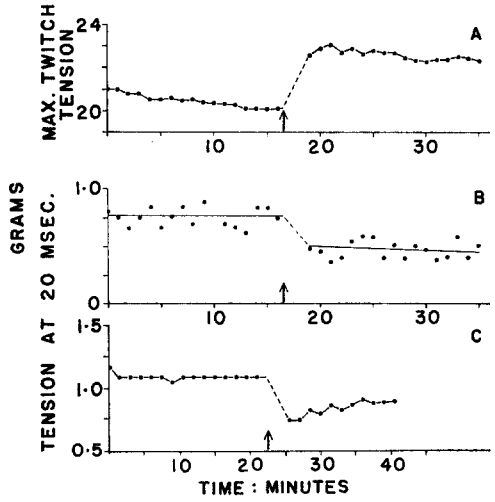

FIGURE 5. The effect of nitrate upon maximum twitch tension and the tension developed 20 msec. after the stimulus. Recordings were made at regular intervals first in chloride-Ringer and then (after arrow) in nitrate-Ringer solution. Records in *A* and *B* were obtained from the same muscle at 1.5°C; those in *C* were obtained from another muscle at 1.0°C. Muscles at rest length.

response to a second stimulus given while the muscle was relaxing about 600 msec. after the first stimulus. The latent period after the first stimulus was no greater than 10 msec. Following the second stimulus the tension continued to decrease for about 17 msec. at which time the tension:time curve passed through a minimum and subsequently increased. The time at which the tension:time curve for the second response diverged from that for relaxation following a single stimulus, was indicated by a sudden decrease in the rate of decrease of tension shortly after the second stimulus. This change occurred about 10 msec. after the second stimulus irrespective of the time at which the second stimulus was given after the first.

The dashed lines in Fig. 3 have been drawn to indicate the rates of rise of tension at the same tension in the two responses. In the second response at 40 msec. the rate of rise of tension was equal to that in the twitch at the same tension at 65 msec. At 30 msec., or at any other time before 40 msec., the rate of rise of tension in the second response was less than that in the twitch at the same tension.

Effects of Nitrate on the Isometric Twitch

Fig. 4 is composed of two superimposed records; one is a twitch in chloride-Ringer, the other a twitch in nitrate-Ringer. In each case the early part of the twitch (modulated trace) and the greater part of the twitch were recorded simultaneously at different sweep speeds and different sensitivities of the vertical amplifiers. The muscle was stimulated at suitable intervals first in chloride-Ringer, then in nitrate-Ringer. The solutions were changed rapidly and care was taken to ensure that both solutions were at the same temperature; in these experiments the temperature of the bath fluid was recorded every minute. The maximum twitch tension in the presence of nitrate was greater and occurred later after the stimulus than in the presence of chloride. Following the initial slower rise of tension in the presence of nitrate the prolongation of the active state was reflected by a crossing-over of the tension: time curves (not shown completely here). A more detailed study of the effect of nitrate on the early phase of contraction showed that at rest length the latent period is not altered by these ions and that it is only during the first millisecond or two after the onset of contraction that the rate of rise of tension at a particular tension is reduced in the presence of nitrate. The effect of nitrate ions on maximum twitch tension, and the tension developed 20 msec. after the stimulus, reached a maximum within 3 minutes after changing the solution (Fig. 5 *A*, *B*). The records shown in Figs. 4, 5 *A*, and 5 *B* were obtained from the same muscle. Fig. 5 *C* shows the results obtained from another muscle and is to be compared with *B* of the same figure. Here again the effect of nitrate reaches a maximum within a few minutes.

DISCUSSION

Time and Degree of Maximum Activation during the Twitch

The differences in the instantaneous rates of rise of tension at any particular tension early in the contraction phases of the twitch and the redevelopment of tetanic tension (Fig. 1) could be due to a progressive change in the properties of either the series elastic elements or the contractile material. Neither of these components has been completely characterized during the early part of the twitch but it has been shown that shortly after the onset of contraction, when the load is very small, these muscles not only shorten isotonically (Hill, 1951 *a*; Abbott and Ritchie, 1951) but also develop tension (Fig. 1) at rates which are about the same as those characteristic of the muscle in the fully active state. If the force:velocity properties of the contractile material are the same in isotonic and isometric contractions this means that there is little or no difference in the properties of the series elastic elements during the early part of the contraction phase of the isometric twitch and during isometric tetanic contractions. This interpretation is consistent with the findings of Wilkie (1956) and Jewell and Wilkie (1958) that in the twitch (after 100 msec.) the series elastic elements are virtually independent of the state of activity of the contractile material. In this case the elastic elements should be extended by the same amount and the contractile component should be at the same length at a particular tension whether the contraction is a twitch or a tetanus. Consequently the differences in the rate of rise of tension such as are evident in Fig. 1 cannot be explained simply on the basis of the relationships between length and tension or length and the speed of shortening of the contractile component. It remains to be considered whether these differences are due to the course of activation of single fibers early in the twitch or whether the redevelopment of tetanic tension is influenced by the effects of release and the previous contraction and as a result is not a true representation of the behavior of the muscle in the fully active state. In this connection it should be pointed out that in these experiments the muscle was released instantaneously and allowed to shorten freely to obviate the effects of slow release upon the redevelopment of tension such as have been described by Abbott and Aubert (1952). The only other situation in which the behavior of the muscle is known to be dependent upon the whole history of the contraction, and which is relevant to the present discussion, is the finding of Ritchie and Wilkie (1958) that for a given load the speed of shortening at certain lengths is dependent upon the initial length. Their records of isotonic contractions beginning at lengths comparable to those before and after release in the present experiments show quite clearly, however, that over the range of lengths from rest length down to at least 88 per cent of rest length, the shortening velocity is the same

in the two cases, being independent not only of the initial length but also of the durations of the contractions. This range of length below rest length is greater than the internal shortening which would take place during the early phase of isometric contractions. Consequently there is no reason to suppose that in the conditions of the present experiments the muscle was not characteristically fully active during the period of redevelopment of isometric tetanic tension following release to a new length. The remaining possibility is that the state of activity of the contractile material is not the same during the early phase of twitch and redeveloped tetanic contractions.

The fully active contractile component should not only shorten at a certain velocity for a given load but also develop tension at a characteristic rate at any particular tension. If the properties of the elastic elements are independent of the state of activity of the contractile material, as has been suggested above, then in particular conditions of load, length, and series elasticity the development of isometric tension at a rate which is lower than that characteristic of the fully active state may be taken as an indication that the contractile component is only partially active; this provides a basis for examining the time course and the degree of activation of the contractile material following a single stimulus. During a brief period, between about 40 and 50 msec. after the stimulus at 0°C, the rate of rise of tension in the muscle is the same during the twitch as it is for the fully active muscle at the same tension (Figs. 1 and 2). In accordance with the above considerations these records can be interpreted as meaning that the contractile component is fully activated in the twitch at 0°C but not until 40 msec. after the stimulus and then only for a few milliseconds. Furthermore, the rate of rise of tension in the second response in Fig. 3 was less at any time before 40 msec. than the rate of rise of tension in the twitch at the same tension. Again this indicates that the contractile component was only partially activated before 40 msec. after the stimulus.

The interpretation of the present results is consistent with the findings of Hill (1949 *b*) and Macpherson and Wilkie (1954) on two points, namely, that these muscles behave as though they are fully activated at about 40 msec. after a single stimulus and that the active state appears to begin to decline shortly thereafter, but it is not in agreement with the conclusions of Hill (1951 *a*), Abbott and Ritchie (1951), or Macpherson and Wilkie (1954) in connection with the amount of time required for the development of the fully active state during the twitch. Macpherson and Wilkie found that the early part of the contraction phase of an isometric twitch was not altered by a second stimulus and they believed this indicated that in the twitch there is a plateau of full activity lasting from the onset of contraction until 40 to 50 msec. after the stimulus. Hill, and Abbott and Ritchie showed that as early as 20 to 25 msec. after a single stimulus these muscles can lift very small loads at nearly the same speed as that which is characteristic of the muscle in the

fully active state and they suggested that this indicated that the contractile component had been fully activated. It should be pointed out, however, that depending upon the pattern of activation of the contractile elements within each sarcomere quite large differences in the degree of activation could be reflected by only small differences in the shortening velocity at low loads. Consider for example, a typical frog sartorius muscle for which the maximum isometric tetanic tension is 50 gm and the force constant (a in Hill's equation) is 12.5. If in every fiber of the muscle only half of the total number of contractile filaments within each sarcomere were fully activated then, according to Hill's equation, the muscle should shorten isotonically against a load equal to 0.5 gm weight at more than 95 per cent of the speed which is characteristic of the muscle in the fully active state. The results obtained by Hill and Abbott and Ritchie do show quite clearly, however, that the speed at which the muscle can lift very small loads is *nearly* the same for tetanic contractions and for shortening occurring at about 20 to 25 msec. after a single stimulus. Any difference in the speed of shortening in these two conditions would be less if the load were smaller and it is fair to conclude that if the load were zero the difference would be negligible. Although this does not indicate that all the contractile material is fully activated it does mean that some of the contractile filaments in each sarcomere are completely activated along their entire length because, in particular experimental conditions, the maximum velocity of shortening when the load is zero is only dependent upon the amount of fully active contractile material in series within the muscle. This interpretation is not inconsistent with the conclusion that the fully active state of the whole contractile component is not developed until about 40 msec. after the stimulus.

Spatial Distribution of Active Elements during the Development of the Active State: Pattern of Activation

The gradation of activity during the course of the development of the active state involves, presumably, a particular spatial distribution of active, or partially active contractile units within the sarcomere. In this connection it is necessary to consider whether only a fraction of the total number of contractile units is involved, each of these units being fully activated, or, whether all the contractile material is partially activated, each unit behaving in a graded fashion. The possible patterns of gradation of activity are numerous but a gross division into three main types may be made as follows:—

(A) SYNCHRONOUS ACTIVATION OF ALL CONTRACTILE UNITS In this case the gradation of activity is entirely temporal. The excitation-contraction coupling mechanism is instantaneous and the time required for the sarcomere to become fully activated is dependent solely upon the rate of an activation process which takes place locally at each contractile unit.

(B) LONGITUDINAL GRADATION OF ACTIVITY Each fibril is supposed to be composed of serially arranged contractile units which during activation are brought into play one after the other in the longitudinal direction. Activation in this sense is supposed to be synchronous in all the fibrils within a sarcomere.
(C) TRANSVERSE GRADATION OF ACTIVITY In this case the time required for a sarcomere to become fully activated is dependent upon the rate of propagation of an excitation-contraction coupling process which travels from the periphery of the fiber to the center. Activation is supposed to occur instantaneously as this disturbance sweeps by each fibril.

The pattern for transverse gradation of activity is the only one of these three which allows some of the fibrils to be completely activated along their entire length before the sarcomere as a whole becomes fully activated. Consequently it is the only pattern of activation which is consistent with the finding that shortly after the onset of contraction the partially active muscle can lift very small loads at virtually the same speed as that which is characteristic of the fully active muscle.

The behavior of the muscle at any time between the onset of contraction and the time at which it appears to become fully active can be compared with the behavior which is predicted from the model for transverse gradation of activity if certain assumptions are made for the purpose of calculation. The experimental data from Fig. 1 have been replotted in Fig. 6 together with theoretical values which were obtained in the following way. It was assumed that (1) the intensity of the active state in a fiber is proportional to the cross-sectional area activated, (2) the intensity:time curve for the development of the active state is a parabola and (3) in all fibers the time required for full development of the active state is 33 msec. (from 9 to 42 msec. after the stimulus in Figs. 1 and 2). Values obtained for the intensity of the active state at various times are given in the legend of Fig. 6. After the first contractile filaments become fully activated along their entire length the model requires that (1) the partially active muscle should obey Hill's characteristic equation and (2) the velocity constant (b) and the ratio of the force constant (a) to the intensity of the active state should be the same whether the muscle is partially or fully active. The force:velocity properties can therefore be calculated for any particular time after the onset of contraction from Hill's equation, the theoretical value for the intensity of the active state and the ratio $a/P_o = 0.3$, which was determined from isotonic tetanic contractions. The ratio of the rates of rise of tension for the partially and fully active states is directly proportional to the ratio of the speeds of isotonic shortening if the properties of the series elastic elements are independent of the state of activity of the contractile material. The rate of rise of twitch tension was predicted on that basis for tensions which were developed at certain times during the twitch (obtained from the tension:time record in Fig. 2) and the values

obtained have been plotted in Fig. 6 for comparison with the observed values. The form of the curve is essentially the same in both cases. One of the most striking similarities illustrated by Fig. 6 is that for both sets of data the ratio of the rate of rise of tension in the twitch to the rate which is characteristic of the muscle in the fully active state is nearly unity at low tensions and then decreases before increasing to unity later in the twitch.

The good agreement between the observed values in Fig. 6 and those calcu-

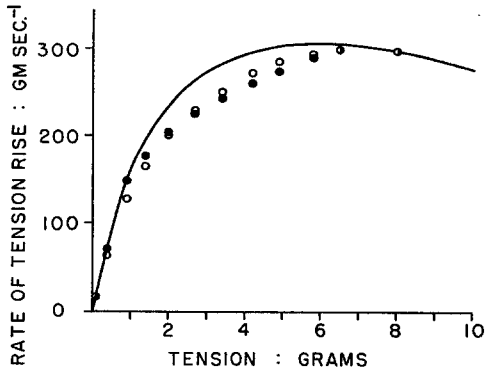

FIGURE 6. Rate of rise of tension:tension for the development of isometric tension in a twitch (observed values ●; calculated values ○) and for the redevelopment of tension in the fully active muscle following release (continuous curve). The observed values for the twitch and the curve for the redevelopment of tetanic tension were obtained from the experimental records shown in Fig. 1. Theoretical values for the twitch were calculated as described in the text. The calculated and observed values for the rates of rise of tension in the twitch are for tensions developed at certain times after the stimulus. For the plotted points, from left to right, these times (milliseconds) and the theoretical values (in parentheses) for the intensity of the active state (calculated as described in the text and expressed as a percentage of the maximum intensity) are as follows: 12.5 (20.2), 15.0 (33.0), 17.5 (44.9), 20.0 (55.6), 22.5 (65.1), 25.0 (73.4), 27.5 (80.7), 30.0 (86.8), 32.5 (91.7), 35.0 (95.5), 37.5 (98.1), 42.0 (100).

lated on the basis of the model for transverse gradation of activity does not prove that this is the pattern of activation though this model does seem to provide a simple explanation for the behavior of the muscle. Furthermore, whereas it was assumed that each contractile filament is activated instantaneously the possibility that this process actually takes some time has not been excluded. If the pattern of activation of the muscle were exactly the same as that described by the model, then the rising phase of the active state curve should be parabolic with the rate of increase of intensity being maximal at the onset. There is some evidence that this is not so and that the active state does not develop at its maximum rate until a few milliseconds after the onset of isometric twitch contraction (Close, 1960). If so much time is required for activation of each contractile filament, then, with regard to the rate at which tension is developed at a particular tension, the behavior of the muscle should

differ from that predicted by the simple model of transverse gradation of activity but only during the period of activation of the most peripheral filaments at the onset of contraction. After complete activation of the peripheral filaments the muscle should behave in the manner predicted by this simple model since the more central filaments, being arranged in parallel with the fully active elements, should not contribute to the development of tension to any significant extent until they become fully activated.

The hypothesis which emerges from the present work is that after the first contractile filaments have become fully activated, further increase in the intensity of the active state is due to an increase in the number of fully active contractile filaments in parallel within the fiber, and that the rate of activation of a sarcomere is determined mainly by the rate at which the transverse component of the link between excitation and contraction is propagated inwards from the periphery to the center of the fiber.

Effects of Nitrate

The retardation of tension development in the presence of nitrate ions occurs in the early part of the contraction phase of the twitch during the period of development of the active state. The records in Fig. 4 show that it is only during the first millisecond or two after the onset of isometric contraction that this effect is seen as a decrease in the rate at which tension is developed at a particular tension. This effect of nitrate could be explained in terms of the pattern of transverse gradation of activity if these ions increased the time required for complete activation of each contractile filament but had little or no effect upon the rate of propagation of the transverse component of excitation-contraction coupling. Such an action should be evident as a lowering of the rate of rise of tension only during the period of activation of the most peripheral filaments shortly after the onset of contraction. Later activation of more central filaments might likewise be retarded in the presence of nitrate ions but, as has been mentioned above, the rate at which the active state appears to develop during this time should be independent of the time required for activation of single filaments and the rate of rise of tension at a given tension should be the same whether the major anion present is chloride or nitrate. If the nitrate alters only the time course of activation of single filaments then, in terms of the pattern of transverse gradation of activity, its action would be mediated through some process which is initiated by the transverse component of the coupling mechanism presumably at sites along its path of propagation. It is possible that the retarding effect is brought about through some action in the endoplasmic reticulum since there is evidence (see below) which suggests that this structure is involved in the propagation of the coupling process. In view of the fact that both the retarding and the potentiating effects become maximal at the same time after changing the

solutions (Fig. 5) it is probable that the two actions take place at the same sites or at nearby sites. In this connection it is interesting to note the suggestion of Hodgkin and Horowicz (1960) that the potentiating effect of nitrate is mediated in some elements of the endoplasmic reticulum.

Hypotheses on the Nature of Excitation-Contraction Coupling

Some of the hypotheses on the nature of excitation-contraction coupling can be considered in relation to the present work since they require particular temporal and spatial distributions of activity in the contractile component of the sarcomere during activation.

Bay, Goodall, and Szent-Györgyi (1953) have suggested that longitudinal action currents activate the contractile elements directly and thereby constitute the link between excitation and contraction. Csapo and Suzuki (1958) have postulated instead that an activating substance is transported by these internal currents. These two hypotheses require "synchronous activation of all contractile units" in the sarcomere and "longitudinal gradation of activity" respectively, but the mechanical behavior of the muscle cannot be explained in terms of these patterns of activation. Moreover the basis for the hypotheses of Bay *et al.* and of Csapo and Suzuki is not consistent with the results of experiments performed by Sten-Knudsen (1954), Watanabe (1958), Buchthal and Sten-Knudsen (1959), and Sten-Knudsen (1960).

Sandow, in his review of excitation-contraction coupling (1952), proposed that an activating substance, such as calcium, is liberated at the membrane and transported to the center of the fiber by a kind of exchange diffusion between adjacent myofibrils. Such a mechanism is conceivable in terms of the present work since it would require time for inward spread in the transverse direction but, as Huxley (1957) has pointed out, this mechanism would probably be self-propagating and it would be difficult to reconcile this with the graded contractions which have been recorded (Gelfan, 1933; Brown and Sichel, 1936; Sichel and Prosser, 1940; Watanabe, 1958; Huxley, 1959).

Porter and Palade (1957) and, as they pointed out, others before them, have suggested that the endoplasmic reticulum might be concerned in the inward spread of the process which couples membrane excitation and contraction. Huxley and his coworkers (see Huxley, 1959) have shown that local activation with microelectrodes can be achieved only when the electrode is at certain positions along the sarcomere and that these positions correspond to the distribution of the transversely arranged triads of the endoplasmic reticulum. The working hypothesis of Huxley (1959) is that the mechanism of inward spread is electrical and occurs along some continuous tubular component. If this occurs along the endoplasmic reticulum and if the propagation velocity is the same throughout then, in view of the arrangement of this structure, more time would be required for inward spread in the trans-

verse direction than in the longitudinal direction. In this case the peripheral fibrils would be activated earlier than those near the center of the fiber and the pattern of gradation of activity would be essentially the same as that envisaged in the present work on the basis of the mechanical behavior of the muscle.

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