

## THE DURATION OF THE ACTIVE STATE IN A MUSCLE TWITCH

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In an isometric tetanus the tension takes an appreciable time to reach its maximum value because of the presence of the series elastic component. In a twitch the initial physical state is the same as it is in a tetanus, but this active state is maintained at its full level for only a short time. The peak tension reached in the twitch will thus depend on the duration of the active state—the longer this duration, the more closely will the twitch tension approach the tetanic tension. Many different treatments are known to alter the twitch tension without appreciable effect on the tetanic tension (e.g. change in temperature, hydrostatic pressure; application of adrenaline, potassium, caffeine, etc.; see Goffart & Ritchie, 1952), and it may well be that these treatments produce their effect by altering the duration of the active state without any other change in the properties of the fully active muscle.

Measurements of the duration of the fully active state have already been made by stretching (Hill, 1949, 1951) or releasing (Hill, 1953) a muscle during a twitch, meanwhile recording its tension. In either case the end of the fully active state is indicated by a decline in the tension record; and by both methods this was found to occur about 90 msec after the stimulus (frog sartorius, 0° C).

The new method to be described here (see also Macpherson & Wilkie, 1953) was evolved in order to make measurements of the duration of the active state at high hydrostatic pressure, so the apparatus had to fit into a small volume. However, the method is of general interest because it has turned out to be more sensitive and more flexible than the previous ones.

The principle of the method is illustrated by Fig. 1, which shows how the tension rises in an isometric tetanus (curve *A*), and in an isometric twitch (curve *B*). During the early part of the twitch the muscle is fully active, so it behaves exactly as though it were tetanized. The early part of curve *B* therefore coincides with curve *A*; and the point at which the two curves separate (about 70 msec after the stimulus) indicates the beginning of the decline of the

active state. In experimental practice it is best not to tetanize the muscle but prolong the active state by giving a second shock so timed that the plateau in the active state is smoothly maintained (curves *C*). Enough of the 'tetanic' curve is thus made available for the point of separation from the single twitch curve to be clearly visible.

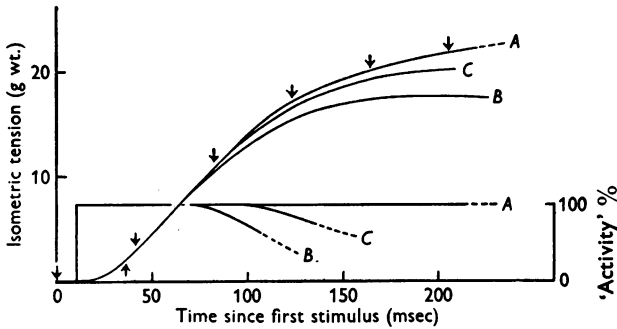


Fig. 1. Curves of isometric tension (left ordinate) and 'activity' (right ordinate) during the early stages of contraction. Frog sartorius, 0° C. Stimuli indicated by arrows: *A*, tetanus; *B* twitch; *C*, summated response to two stimuli.

#### EXPERIMENTAL TECHNIQUE

A single sartorius from a frog (*Rana temporaria*) was mounted at its body-length on a multi-electrode apparatus (Hill, 1949) and stimulated in oxygenated Ringer's fluid with supramaximal rectangular pulses of duration 0.2 msec (approximately equal to the chronaxie). The muscle was not curarized, so its fibres were stimulated in part directly, in part through intramuscular nerve twigs. Tension was registered by a mechano-electronic transducer (RCA 5734) whose output was displayed on a cathode-ray tube and recorded by a still quarter-plate camera. There was no need for conditions to be very strictly isometric so the muscle was connected to the transducer by a length of plaited silk braid which had been oiled to make it hang limply. With this form of connexion vibrations were never a serious problem. Triggering of time-base and stimulator was controlled by a modified Keith Lucas rotary contact breaker, electrically driven and built from standard gramophone parts.

Records like those in Figs. 1-3 were built up by recording several contractions on the same piece of photographic paper. If the traces are to superimpose accurately both the apparatus and the muscle must be in a very stable condition, and the triggering arrangements must be consistent to a small fraction of a millisecond. Accurately reproducible twitches were obtained by stimulating the muscle automatically every 3 min. In this way the metabolism is soon brought to a steady level and no difference can be detected between successive twitches.

#### RESULTS

A typical experimental record is illustrated in Fig. 2, which shows the active state produced by the first shock beginning to decline after about 70 msec. Records *B* and *C* run together for an appreciable time, proving that the timing of the second shock can be varied over quite a large range (30 msec in this case) without producing an appreciable departure from the condition of full activity, and substantiating the belief that the active state curve has a really flat

plateau. If the second shock is given later still (e.g. at *D*), the activity resulting from the first shock does have time to decay before being replenished by the second shock. The resulting tension curve *D* therefore follows at first curve *A* rather than curves *B* and *C*. Both for first and for subsequent shocks there is a latency of about 15 msec before any effect on the tension curve can be detected. However, the measurements of the duration of the fully active state given in this paper are all made *from the first shock* and *not* from the end of this latent period.

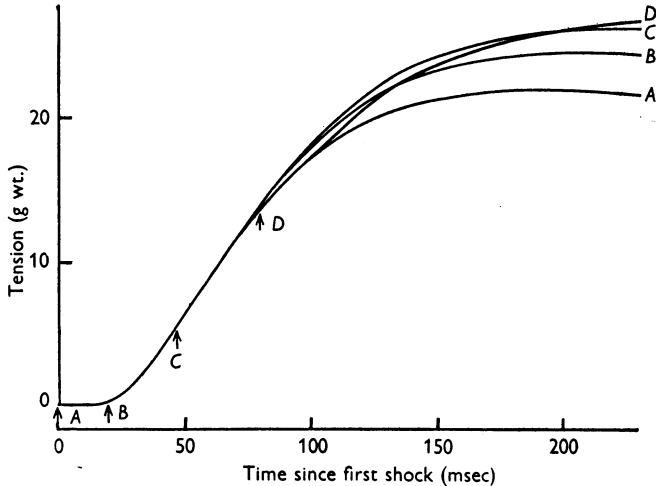


Fig. 2. The rise in isometric tension during the early stages of contraction. Single frog's sartorius, 0° C; tracings. *A*, response to single stimulus at time zero; *B*, response to pair of stimuli, the second being given at time *B*; *C*, response to pair of stimuli, the second being given at time *C*; *D*, response to pair of stimuli, the second being given at time *D*.

*Differentiated records.* The exact moment at which curves *B* and *C* can be seen to separate from curve *A* naturally depends on the resolution of the method employed to examine the region of separation. This resolution can be improved by differentiating the tension record, performed experimentally by passing the output from the transducer through a series resistance-capacitance circuit ( $RC = 3$  msec) and increasing the amplifier gain appropriately. A record obtained in this way is shown in Fig. 3: the peak on this curve corresponds to the point of inflexion in Fig. 2. Curves *A*–*D* show qualitatively the same relationship as in Fig. 2; but, as expected, the times involved are all shorter. Whether or not the apparent duration of the active state would be still further reduced by further increase in resolution is a question which cannot be answered theoretically without foreknowledge of the exact shape of the active state curve; but it seems probable that further improvement in technique would yield only diminishing returns.

*Numerical results.* The numerical results obtained by the two methods are given in Table 1.

*Variation with temperature.* We have confirmed the finding of Hill (1951) that the duration of the active state is greatly reduced by an increase in

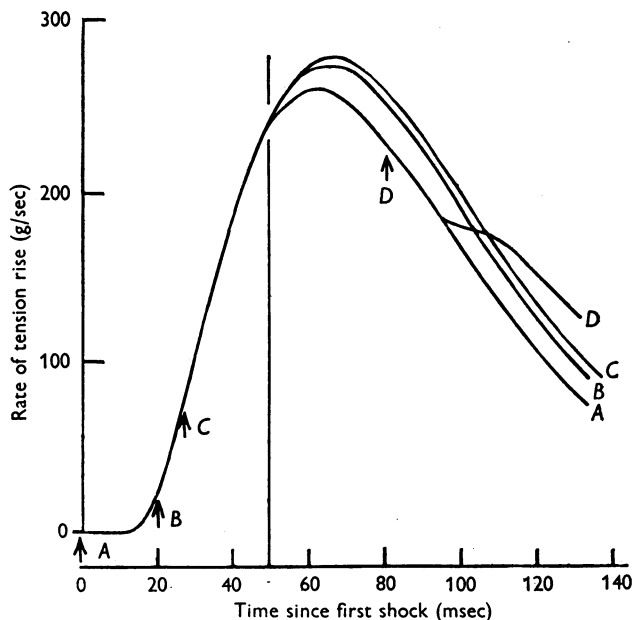


Fig. 3. Rate-of-change of isometric tension,  $P$ , against time. Frog's sartorius,  $0^{\circ}\text{C}$ ; tracings A-D have the same significance as in Fig. 2. End of plateau indicated by vertical line.

TABLE 1. Duration of the plateau of the active state. Frog's sartorius,  $0^{\circ}\text{C}$  (June and July); mean  $\pm$  standard deviation about mean.

Method	Duration (msec)	
Tension record	$65 \pm 12$	(8 determinations on 2 muscles)
Differential	$44 \pm 5$	(11 determinations on 5 muscles)

All measurements made with a stimulus strength about 15 times threshold. Times measured from the first shock.

temperature. The effect is demonstrated quantitatively in Fig. 4 (solid circles), where it will be seen that the duration of the active state is approximately halved by each  $10^{\circ}\text{C}$  rise in temperature.

*Variation with stimulus strength.* Fig. 4 also reveals that the duration of the fully active state varies somewhat with the strength of the stimulus, particularly at low temperature. That all the stimuli used were supramaximal in the ordinary sense is shown by the open circles in Fig. 4, which show how the peak height of the twitch, on which the assessment of maximality is commonly based, varied

with the stimulus strength. The upper part of this curve is sometimes perfectly horizontal (as in the muscle of Fig. 5 B) but more often it slopes gently upwards. This is not unexpected, since the height of the twitch must depend in a complicated way on the duration and on the mode of decline of the active state; while the duration demonstrably varies with the stimulus strength.

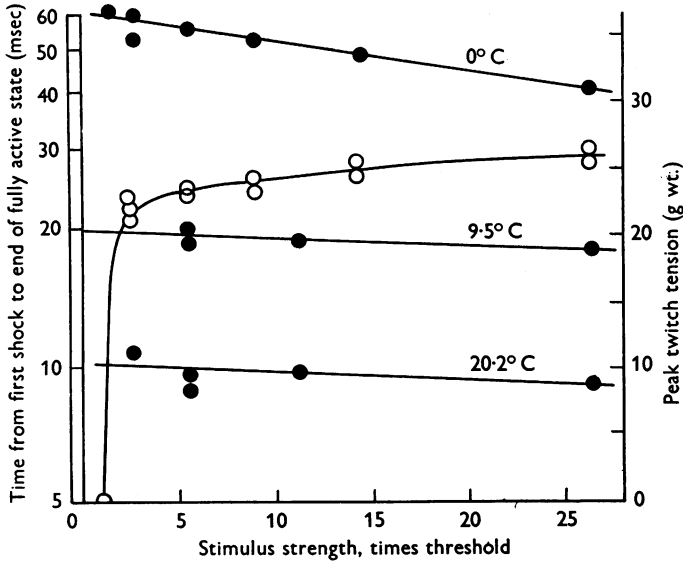


Fig. 4. Solid circles: time from first shock to end of the fully active state at various stimulus strengths, and at the different temperatures indicated on the curves (left ordinate). Open circles: peak height of twitch in g weight (right ordinate).

Why does the duration of the active state vary with stimulus strength? Part of the effect must, and all of the effect may, be due to the following cause: that every part of the muscle is not stimulated simultaneously even on the multi-electrode assembly. The alternate anodes and cathodes are spaced 3 mm apart, and if a threshold shock stimulates only in the immediate neighbourhood of a cathode the remainder of the muscle must be stimulated by conduction of impulses along nerve twigs and muscle fibres. Muscle lying over the anodes will therefore not become active until the impulses have travelled 3 mm, which would take about 10 msec in muscle fibres at 0°C. The effect of this asynchrony on the active state curve of the whole muscle will be to prolong both its rise and its fall. The prolongation must be diminished by raising the stimulus strength, for then more and more of the muscle will be stimulated directly, rather than by conduction from other parts. A close connexion between this effect and the impulse conduction velocity may be inferred from Fig. 4. The

effect of stimulus strength on the duration of the active state practically disappears at 20° C, presumably because all the conduction processes take place much more quickly at the higher temperature. Certainly the time-course of isometric tension development is altered by altering the (supramaximal) stimulus strength, as shown in Fig. 5 A; and the difference persists throughout the whole of the rising phase of the twitch. Does this mean that the nature of the active state depends on the stimulus strength, or can the effect be accounted for by a difference in the time-course of the rise and decay of activity?

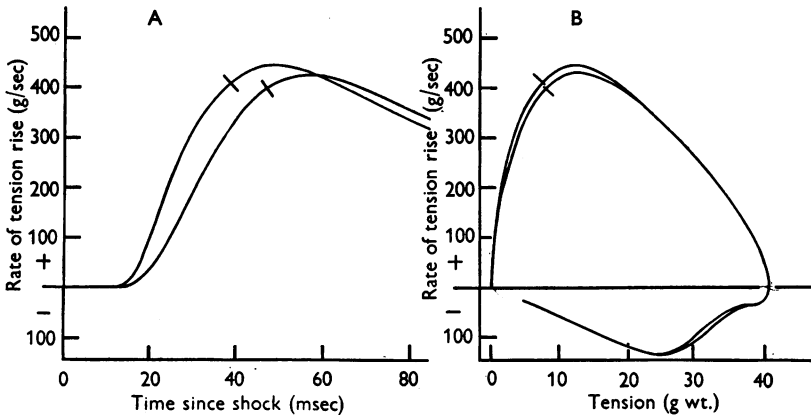


Fig. 5. The effect of stimulus strength on the onset of contraction. In both A and B the upper curve is a response to a single shock of 20 times threshold, the lower to a shock of 5 times threshold, given at time zero. The bar indicates the end of the plateau in the active state, determined from other experiments. A,  $dP/dt$  displayed against time  $t$  (as in Fig. 3); B,  $dP/dt$  displayed against tension  $P$ .

This question can be investigated by displaying  $dP/dt$  as a function of tension  $P$  as in Fig. 5 B, rather than of time as in Fig. 5 A. This may easily be carried out experimentally by feeding the X plates of the cathode-ray tube from the transducer instead of from the time-base. It can be shown that at a given instant the height of the  $dP/dt: t$  curve depends on past history; that is, on the shape of the curve at previous times. On the other hand, the position of each point on the  $dP/dt: P$  curve depends solely on the condition of the muscle at that instant.

The time-course of isometric tension development is known to be determined by the force-velocity relation in the fully active contractile element and the force-extension relation in the elastic element. Thus at a given tension,  $P$ , the velocity of shortening,  $v$ , and the series compliance,  $C$ , are both fixed.  $dP/dt$  is also fixed, for  $v = CdP/dt$ .  $dP/dt$  is therefore a fixed function of  $P$  all the time that the active state is fully maintained.

The close similarity between the curves in Fig. 5 B therefore provides direct evidence that change in stimulating voltage has very little effect on the nature

of the active state. It therefore seems reasonable to conclude that the stimulating voltage alters tension development mainly or solely by altering the time-course of the rise and decay of activity.

The  $dP/dt:P$  curve has several interesting properties which may make it useful in the study of muscular function. Apart from its present use to detect full activity, the curve may be employed to reveal regions of exponential rise or fall of tension, which appear as straight lines through the origin (O. Sten-Knudsen, private communication); while the method of calculating muscle constants from a pair of isometric contractions with and without added compliance (Macpherson, 1953), is greatly facilitated. If desired, time may be recorded as third variable by modulating the cathode beam.

#### DISCUSSION

The reasoning on which this method is based assumes implicitly that the second shock is given after the refractory period from the first shock has substantially passed, so that the excitatory mechanism is fired off twice. The numerical values for the decay of refractoriness at different temperatures given by Adrian (1921) show that this assumption is justified.

The times given here for the duration of the plateau of the active state are considerably shorter than those given by Hill (1949, 1953) because of the greater sensitivity with which a decline from full activity can be detected; and it is interesting to note that our figures agree quite well with measurements of the duration of the active state generated by each shock during a fully-developed tetanus (Hill & Macpherson, 1952, referred to by Hill, 1953, p. 502; Mauriello & Sandow, 1953; Ritchie, 1954). These measurements were made by finding the lowest frequency of stimulation at which no tension fluctuations could be detected, or by measuring how soon after the last shock the tension just began to fall. It should be noted that the latter method measures the interval from the shock to the first decline in the active state, while the former measures this interval *minus* the latent period.

Measurements of the active state are important because they reflect directly the processes going on in the contractile element of the muscle. In contrast, the more commonly measured isometric twitch tension reveals these processes very indirectly, for the shape and size of the twitch depend in a complex way on the shape of the active state curve and on the compliance of the series elastic element and any external connexions. To understand more fully what happens in a twitch it will be necessary to examine the mechanical properties of the contractile element during the period when the active state is declining, for much of the tension rise occurs during this period. It would be very interesting to find out whether there is still a relation between force and velocity of shortening, and one might learn much from the way in which the contractile machinery is dismantled. During relaxation, when one part of the muscle is

stretching another, conditions must be still more complex. We hope to extend this work by studying contraction under high hydrostatic pressure, which is known to cause large changes in the size and shape of twitches, possibly as a result of corresponding changes in the active state curve.

## SUMMARY

1. The duration of the 'plateau' of the active state in a muscle twitch has been measured by comparing the curves of isometric tension rise in a twitch and in a tetanus. The end of the plateau is marked by a separation of the curves.

2. The plateau ended  $44 \pm 5$  msec (mean  $\pm$  s.d.) after the stimulus in frog's sartorii at  $0^\circ$  C.

3. The effects on the active state of temperature and of stimulus strength have been investigated.

We wish to express here our thanks to Prof. A. V. Hill for a number of valuable suggestions about the conduct of these experiments.

We understand that Dr A. Sandow, of New York University, has recently investigated the time-course of the active state by a method similar to the one described here. He has obtained results similar to ours.

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