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THE EFFECT OF PREVIOUS STIMULATION ON THE ACTIVE STATE OF MUSCLE

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Recently one of us (Ritchie, 1954*b*) has described a method for determining the intensity of activity in a muscle at any instant after a single stimulus. The intensity of activity (or 'of the active state') is defined as the tension which the contractile component of the muscle would exert if it were neither lengthening nor shortening. However, the contractile component is seldom in this condition, for even when the ends of a muscle are fixed, as in ordinary isometric recording, one cannot prevent the contractile component from altering in length; as the tension changes, the length of the series elastic component also changes. Only when the rate of change of tension is zero—for example at the peak of an isometric twitch—are both the elastic component and the contractile component at unchanging length. In this situation, therefore, the peak tension exerted by the whole muscle must be equal to the intensity of activity as defined above.

This reasoning forms the basis of the method for determining the intensity of activity. A frog's muscle is arranged to be stretched 3-5 mm beyond the final length at which the measurements are to be made. A single maximal shock causes it to contract isometrically; then, at a pre-set moment, the muscle is released, its tension falls to zero, and the redevelopment of tension at the final length is recorded. This redevelopment of tension corresponds to the quick-release phenomenon of Gasser & Hill (1924). The release allows the undamped elastic component to revert suddenly to its unstretched condition. The contractile component responds instantly to the fall in tension, but it can only shorten at a limited rate, so the redevelopment of tension is correspondingly slow. No change appears to be produced in the contractile component as a direct result of the release (Hill, 1953). There is no sign of any change in intrinsic properties corresponding to that found by Pringle (1954) on sudden release of a cicada muscle.

The quick-release phenomenon following a single shock is of importance for our purpose, in that by varying the moment (and/or the amount) of release, it is possible to record a set of tension curves whose peaks lie at different tensions. The line joining the peaks of these records traces out the active state curve.

So far the active state curve has only been determined using single stimuli. In the present experiments the curve was determined using multiple stimuli for the following reasons. If previous stimulation were to prolong the duration of the active state, two well-known phenomena, the staircase effect and post-tetanic potentiation, could be explained in terms of changes of the active state. Furthermore, by using multiple stimulation the whole of the active state curve can be obtained. The use of single stimuli gives the curve only from the peak twitch tension downwards, i.e. only the bottom two-thirds of the curve, even in the favourable case of a frog's muscle at 0° C. To obtain the full curve it has to be measured from the maximum tetanic tension downwards.

METHODS

The technique employed was similar to that described by Ritchie (1954*b*). All experiments were performed at 0° C on the frog's sartorius muscle suspended in oxygenated Ringer's solution (NaCl, 115.5 mM; KCl, 2.0 mM; CaCl₂, 1.8 or 3.6 mM; phosphate buffer, pH 7.0, 2.0 mM), stimulated by means of a multi-electrode assembly (Hill, 1949) and connected by a thin straight wire of malleable stainless steel to the tip of a light duralumin lever. The lever was prevented from moving by a stop which could be suddenly withdrawn by breaking the current through an electromagnet. Above the lever was a mechano-electronic transducer valve (RCA 5734) connected to the lever tip by a short length of plaited silk. By varying the amount of slack in this thread it was possible to vary the amount of release allowed before recording of the isometric tension began. The compliance in the system was kept as small as possible so that the tension in the elastic component was always reduced momentarily to zero by the release.

The experiments were performed on two separate sets of apparatus and differed slightly in such details as the arrangement of the multiple electrodes, the shape of the stimulus pulse, the geometrical arrangement of the various mechanical parts, and the presence or absence of tubocurarine chloride (1:50,000, w/v). These differences did not lead to any difference in the results obtained.

Care was taken to ensure that the muscle was in a steady condition by stimulating it automatically once per minute throughout each experiment, starting some time before the first record was taken. After each tetanus at least six routine twitches were given to allow the muscle to come back to its steady state, and the number of tetani was kept to a minimum.

RESULTS

The effect of varying the number of stimuli. The following routine was adopted. The muscle was stimulated by a single shock once a minute; by varying the time interval between stimulus and release a number of tension curves were obtained superimposed on the same photographic record, as shown in Fig. 1. The line joining the peaks of these curves is the active state curve and is indicated by the solid circles in Fig. 2. The open circles and the crosses in Fig. 2 were obtained in the same way, except that the muscle had been stimulated

by two and five shocks respectively, the interval between the shocks being 135 msec. It is seen that the three sets of points lie close to the same curve, although the points obtained after multiple stimulation lie slightly to the right; after ten shocks (open triangles) the effect is much more striking. This means that previous stimulation makes the activity last longer.

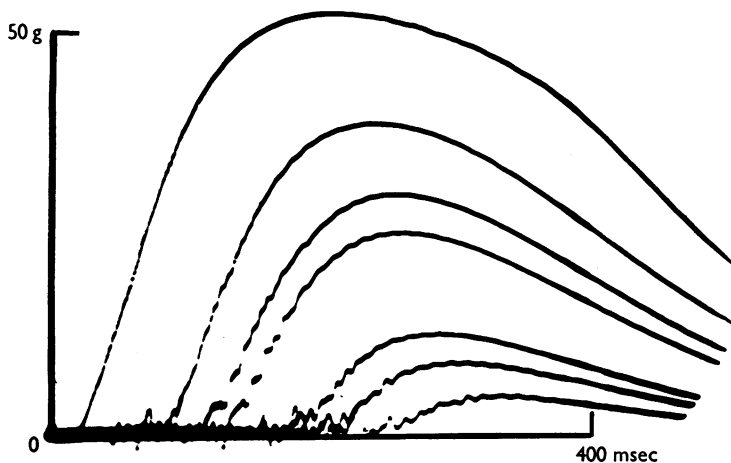


Fig. 1. Records of tension development of frog's sartorius following quick releases at different times during single twitches. The stimulus coincides with the beginning of the record.

A similar shift of the active state curve to the right was obtained when this curve was determined a short time after a tetanus had been given to the muscle. In these experiments tension records were made of the response to single shocks 60 sec *before* and 20 sec *after* a brief tetanus, the amount and time of release being the same for both records. The time of release was then altered and a new pair of records obtained; this was repeated for several different times of release. Between each pair of records the muscle was allowed 6 min or more in which to get back to its steady state. The result of such an experiment is shown in Fig. 3. The general result is the same as that in Fig. 2 and shows, with the slight difference in procedure, that the effect of a tetanus can still be detected after 20 sec. Further, in this experiment the prolongation produced by previous stimulation cannot have been the result of a higher tension existing in the muscle at the moment of release, as might be argued for the experiment of Fig. 2.

In the present experiments the duration of the plateau of the active state was not measured, but this is also prolonged by previous stimulation (Ritchie, 1954*a*).

The staircase effect and post-tetanic potentiation. The essential feature of both phenomena is that previous stimulation increases the peak tension developed in a subsequent muscle twitch. With low frequencies of previous stimulation,

leading to a series of twitches, the result is called the staircase phenomenon: with high frequencies of previous stimulation, leading to a tetanus, the result is called post-tetanic potentiation (Rosenblueth & Morison, 1937; Guttman, Horton & Wilber, 1937; Brown & von Euler, 1938). Both phenomena vary a great deal from one type of muscle to another and are sensitive to slight changes in experimental conditions.

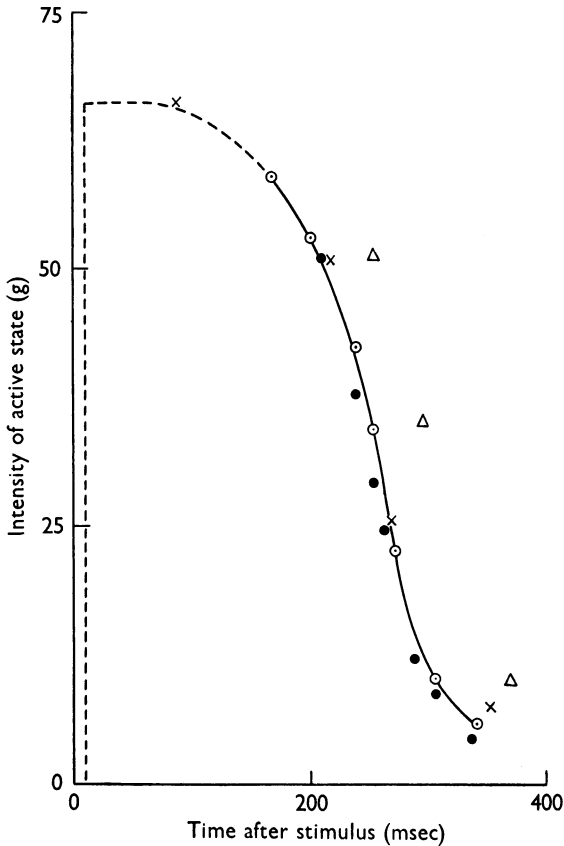


Fig. 2. The decline in activity of frog's sartorius at 0°C following 1 shock (solid circles), 2 shocks (open circles), 5 shocks (crosses), and 10 shocks (triangles). Interval between shocks 135 msec. Times are measured from the last shock in each case. The curve is that obtained using two shocks as the stimulus.

The staircase effect and post-tetanic potentiation can be predicted from the results shown in Figs. 2 and 3, because if previous stimulation makes the active state last longer, it is only reasonable to expect that a higher twitch tension will then be reached, assuming that other properties, e.g. the force-velocity relation, remain constant. The increase in twitch tension of the frog's sartorius

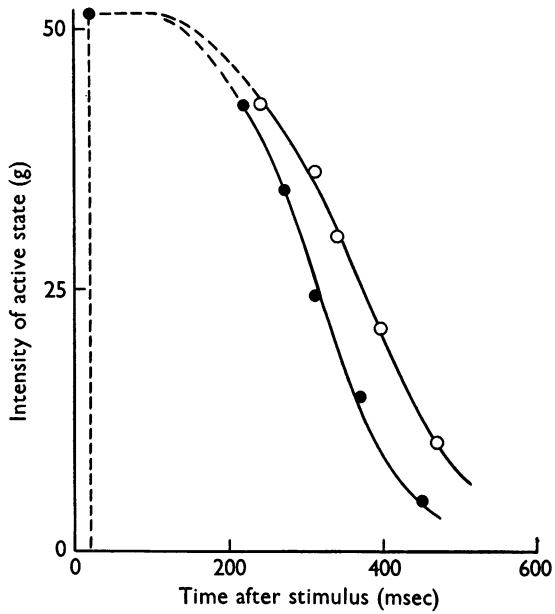


Fig. 3. Active state curves of frog's sartorius at 0°C plotted from single shocks given 60 sec *before* (solid circles) and 20 sec *after* (open circles) a short tetanus (1 sec duration, 50 shocks/sec).

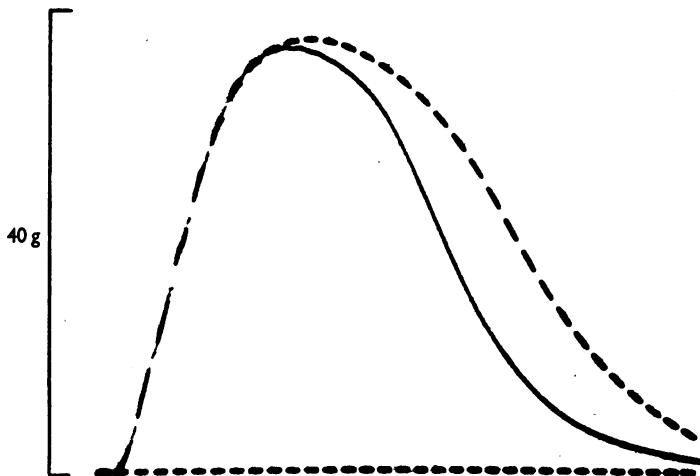


Fig. 4. Isometric twitches of frog's sartorius at 0°C recorded at body length. The first (solid line) and eleventh (interrupted line) of a series of twitches at the rate of 20/min. Time marks 20 msec.

at 0° C after a previous series of twitches is shown in Fig. 4. The effect is not very dramatic, for the twitch:tetanus ratio is large (0.65–0.8) and at the peak of an ordinary isometric twitch the activity curve has fallen only a little. As a result of the previous stimulation the muscle does remain in its active state for a short extra period of time, but this occurs at a moment when the isometric tension is rising only very slowly. The peak tension reached is therefore not much increased, though relaxation is slowed.

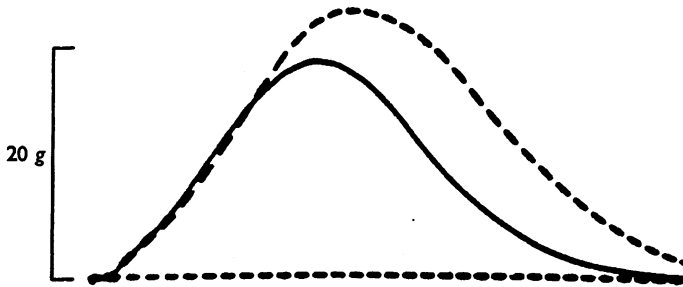


Fig. 5. Same muscle as in Fig. 4. Tension recorded through a compliant connexion (see text). As in Fig. 4, the first (solid line) and eleventh (interrupted line) of a series of twitches at the rate of 20/min.

We now have a fair understanding of the factors underlying the size and shape of a muscle twitch, so that by altering the mechanical system against which the muscle pulls we can arrange for the tension still to be rising at a time when the activity is declining rapidly (see Hill, 1951). In this situation one would expect the peak tension to be far more sensitive to the duration of activity. This conclusion is borne out by experiments in which tension development was delayed by attaching the muscle to the transducer through a compliant instead of an unyielding connexion. The effect is best shown if the compliance is non-linear: in practice we used either another (unstimulated) sartorius or a rubber band whose total stretch was limited by joining it in parallel with a piece of thread. Tension records obtained in this way are shown in Fig. 5; the increase in peak tension is much more dramatic than in Fig. 4.

In both Figs. 4 and 5, the two curves run together for the first 180 msec, which means that previous stimulation alters the time course of activity without affecting the force-velocity curve.

DISCUSSION

Our results show that the active state curve obtained using one shock as the stimulus differs very little from that obtained using two shocks, though the latter extends over a greater range than the former. By giving two shocks so timed that the second falls just before peak tension is produced by the first, it is easy to plot most of the falling part of the active-state curve, even when

the twitch: tetanus ratio is small, as in frog's muscle at room temperature or in mammalian muscle. The need for such a technique was, in fact, our reason for starting these experiments. In other words, our experiments have shown that the activity (in the contractile material) produced by one shock does not summate with that produced by a preceding shock in the way that muscle tension summates. Every shock restores the intensity of the active state to a *maximum* from which it then decays, always with much the *same* time course; so that in a tetanus the phase of falling activity is almost entirely determined by the last shock given to the muscle.

Evidence has been accumulating for some time that many of the agents which are known to increase the peak tension of the isometric twitch do so by prolonging the active state. This explanation certainly applies to the actions of drugs such as adrenaline, caffeine, quinine, nitrate, bromide, iodide and potassium (Goffart & Ritchie, 1952; Hill & Macpherson, 1954; Ritchie, 1954*b*; Lammers & Ritchie, 1955) as well as to the effect of applying high hydrostatic pressures (Wilkie, unpublished). The experiments reported here on frog's muscle have shown that a similar prolongation of the active state can account for the staircase effect and for post-tetanic potentiation. These effects are shown far more dramatically by mammalian muscles, and it would be interesting to know whether or not in these muscles the whole effect, or only part of it, can be accounted for by a change in the activity curve.

Striped muscle is used quite commonly as a pharmacological test object. It is customary to record its isometric tension development on such a slow time base that the peak tension reached is the only significant feature of the record. However, an effect on peak twitch tension does not always give a clear indication of the nature or extent of an action of a drug or treatment on muscle (for example see Fig. 4) for the factors determining this peak tension are complex. The mechanical properties of the recording arrangement and the amount of compliance and slackness in the muscle attachments profoundly influence the result obtained, even if the isometric recorder itself has a sufficiently high frequency response to avoid distortion of the tension curve. Moreover, it is possible by altering the mechanical arrangements to 'amplify' the effect one is seeking (compare Figs. 4 and 5). This is a useful technical trick and it emphasizes the fact that measurements of muscle twitches can only be interpreted quantitatively if the mechanical conditions of recording are clearly defined.

The significance of the active state curve. The technique described here certainly measures 'activity' according to our strict definition of it, and when the experiment is done on a muscle at a constant final length the curve of the active state we obtain turns out to be a single-valued function of the time since the last stimulus. It is independent of the total tension reached and of the amount of release. As a corollary of this we have obtained the same curve

either by allowing a fixed amount of release at varying times, or by varying the amount of release at a fixed time. However, the curve does show a slight dependence on the final muscle length at which it is measured; the shorter the muscle, the earlier the fall (Ritchie, 1954*b*).

The active state curve seems to reflect some process going on in the contracting muscle, but does it tell us anything about the actual mechanism of contraction? On a macroscopic scale the shape of the curve is certainly of importance in determining mechanical behaviour in a muscle twitch; for we have other evidence that the activity at each instant determines not only the maximum isometric tension available, but also the speed of shortening. This makes it possible to predict the mechanical behaviour of the muscle throughout its twitch. On a purely descriptive plane, therefore, the active state curve is an important determinant of muscular function. What this means on a molecular scale in terms of the ultimate contractile mechanism is not clear. One can only speculate, and the facts themselves can probably be accommodated by any of the current theories of the contractile process.

The active state curve is fitted quite well by a cumulative normal probability curve, so the behaviour of the whole muscle may be determined by all-or-nothing subunits arranged in parallel, whose duration of activity is more or less normally distributed. On the other hand, the contractile units may be dismantled individually in a way that follows the activity curve. This could come about for a reason local to each unit, e.g. the exhaustion of a local energy store; or because of some influence pervading the whole of the fibre and affecting all the contractile units together. Perhaps the negative after-potential acts in this way.

SUMMARY

1. The intensity of the active state of frog's muscle has been measured after stimulation by single and by multiple shocks.
2. Previous stimulation tends to delay the fall in activity.
3. This delay is small when only a few shocks are given to the muscle within a short interval of time. Almost all of the declining phase of the active state curve can therefore be determined by using a stimulus which consists of two shocks.
4. The delay in the fall of activity after previous stimulation accounts for the staircase phenomenon and post-tetanic potentiation in frog's muscle.

REFERENCES

- BROWN, G. L. & EULER, U. S. VON (1938). The after effects of a tetanus on mammalian muscle. *J. Physiol.* **93**, 39-60.
- GASSER, H. S. & HILL, A. V. (1924). The dynamics of muscular contraction. *Proc. Roy. Soc. B.* **96**, 398-437.
- GOFFART, M. & RITCHIE, J. M. (1952). The effect of adrenaline on the contraction of mammalian skeletal muscle. *J. Physiol.* **116**, 357-371.

- GUTTMAN, S. A., HORTON, R. G. & WILBER, D. T. (1937). Enhancement of muscle contraction after tetanus. *Amer. J. Physiol.* **119**, 463-473.
- HILL, A. V. (1949). The abrupt transition from rest to activity in muscle. *Proc. Roy. Soc. B*, **136**, 399-420.
- HILL, A. V. (1951). The effect of series compliance on the tension developed in a muscle twitch. *Proc. Roy. Soc. B*, **138**, 325-329.
- HILL, A. V. (1953). The mechanics of active muscle. *Proc. Roy. Soc. B*, **141**, 104-117.
- HILL, A. V. & MACPHERSON, L. (1954). The effect of nitrate iodide and bromide on the duration of the active state in skeletal muscle. *Proc. Roy. Soc. B*, **143**, 81-102.
- LAMMERS, W. & RITCHIE, J. M. (1955). The action of quinine and quinidine on the contractions of striated muscle. *J. Physiol.* **129**, 412-423.
- PRINGLE, J. W. S. (1954). The mechanism of the myogenic rhythm of certain insect striated muscles. *J. Physiol.* **124**, 269-291.
- RITCHIE, J. M. (1954*a*). The duration of the plateau of full activity in frog muscle. *J. Physiol.* **124**, 605-612.
- RITCHIE, J. M. (1954*b*). The effect of nitrate on the active state of muscle. *J. Physiol.* **126**, 155-168.
- ROSENBLUETH, A. & MORISON, R. S. (1937). Curarization, fatigue and Wedensky inhibition. *Amer. J. Physiol.* **119**, 236-256.