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Muscle Twitch Tension—Influence of Electrical Stimulating Conditions at Different Temperatures

I. Results for Chloride Ringer's

By Elizabeth Kelly, William J. Fry and Francis J. Fry
Biophysical Research Laboratory, University of Illinois, Urbana, Illinois

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§ 1. Introduction

Investigations of basic mechanisms of muscle contraction employ, in many cases, excised uncurarized whole skeletal muscle of frog stimulated by mass electrodes or multi-electrodes. The use of whole muscle in preference to single fibres is now usually justified on the basis that *all* fibres within the muscle can be ' simultaneously ' stimulated to respond. However, early investigators in the field of muscle research focussed considerable attention on whether all fibres within a whole muscle respond to the stimulus. More recently this question has not engendered much interest, primarily because of the general acceptance of the concept of the supermaximal stimulus delivered by a mass electrode or multi-electrode. It is apparently accepted that when the area of the electrodes is greater than the maximum projected area of the muscle and the magnitude of the stimulus current results in ' plateau response ', the conditions are satisfied for simultaneous contraction along the entire length of all fibres. This concept is so well accepted that in many publications only the term ' supermaximal stimulus ' is used to describe the stimulation procedure with no quantitative data indicated for stimulus duration, current density or magnitude of developed tension.

We have conducted a detailed study of the effects of variations in the duration of ' supermaximal ' electrical stimuli on the amplitude of the isometric twitch tension of excised skeletal muscle at temperatures above 20°c. This study was initiated because of our speculations regarding the findings of other investigators that the amplitude of peak isometric twitch tension *decreases* with increasing temperature in the range 0° to 25°c, while for the same range the amplitude of tetanus tension increases with increasing temperature (Hill 1951, Hill and Macpherson 1954). The active state theory ' explains ' this by hypothesizing that the duration of the active state decreases as the temperature increases, so that relaxation offsets contraction before full tension can be reached (Hill 1951, Hill and Macpherson 1954, Macpherson and Wilkie 1954). It appears impossible to correlate this explanation with the findings of Buchthal, Weis-Fogh and Rosenfalck (1957) on whole flight muscle of the locust, namely, that the peak twitch tension amplitude increases with

increasing temperature although, according to Buchthal *et al.*, the " relative decay of the active state is quicker in locust flight muscle than in the skeletal muscle of frog ". In addition, Jewell and Wilkie (1958) found essentially no change in isometric twitch tension of frog sartorius muscle with increasing temperature.

The results obtained on the effect of different conditions of electrical stimulation on the amplitude of twitch tension are of direct interest in considering the well-known phenomenon of increased amplitude of twitch tension in response to substitution of nitrate Ringer's solution for chloride Ringer's. This latter aspect of the research results will be presented in subsequent papers, which will also contain a discussion of the results of Part I. Another paper will be concerned with the effects of muscle tendons and different mechanical connecting attachments, between transducer and muscle, on the amplitude of twitch tension and on the time rate of rise of tension.

§ 2. Instrumentation and General Procedure

The instrumentation for recording the isometric tension of the frog sartorius muscle consists of a photoelectric strain gauge transducer capable of measuring tensions from 0 to 225 g, an oscilloscope, and a camera. The transducer contains a spring with a light gate mounted on one end, the linear displacement of the transducer spring amounting to 0·006 mm per gram of applied tension. Light, partially interrupted by the gate, energizes a pair of photosensitive semiconductors mounted side by side and connected electrically to oppose each other. When a force is applied to the spring, the light gate adds light to one cell as it subtracts it from the other. The voltage output of this system, for the forces applied by frog sartorius muscle, is in the millivolt range. This relatively high voltage output is a distinct advantage because it allows the output of the transducer to be fed directly into an oscilloscope without any intervening preamplifier, which often limits the overall frequency response capability of the system. The oscilloscope used in the present study has a vertical sensitivity of 1·0 mv/cm, a minimum sweep time of 1·0 μsec/cm, and a frequency response flat from 0 to 300 kc/sec. In addition to simply determining the resonant frequency (250 c/sec) by observing the response to shock excitation, a comprehensive test of the frequency response characteristic of the transducer and its associated oscilloscope was made. This was accomplished by driving the transducer with a variable frequency, variable displacement, linear motion device designed for this purpose. This device is capable of driving the transducer at frequencies in the range 0 to 200 c/sec at displacements ranging from 0 to $\pm$1·0 mm from the equilibrium position. As a result of this test, it can be concluded that for the temperature range used in this study, the transducer-oscilloscope system recorded the full tension developed by the muscle without reduction due to frequency response limitations.

In many of the experiments reported in this paper the stimulator employed provided constant voltage pulses capable of supplying currents from 0 to 2 amp when used in conjunction with the mass electrodes consisting of two rectangular gold plates with dimensions of $5 \times 45 \times 0{\cdot}25$ mm. These electrode plates were mounted with the large faces parallel and separated by a distance of 5·0 mm. The muscle hung between the electrode plates with its ' flat ' surfaces approximately parallel to the flat faces of the electrodes. Care was taken that the entire volume of the muscle was within the rectangular volume of solution defined by the two plates. The pulse duration of the stimulus could be varied over a wide range of values but was generally kept within 0·2 to 10 msec. For the earlier work, the stimulating current pulse was rectangular for all pulse durations employed (0·2 to 10 msec) except for a deviation at the initiation of the pulse. This deviation was in the form of an overshoot of only 0·4 μsec duration and it is unlikely that it influenced the data to any appreciable extent. However, for the more recent experiments, a constant current stimulator was designed which provided pulses of accurately controlled and reproducible amplitudes from 0·01 to 1·99 amp. A constant current design was employed so that impedance changes due to electrode surface conditions and polarization would not affect the stimulus conditions at the muscle. The results obtained with constant current stimulation correlated closely with the data obtained when the first stimulator was used. Sixty-five individual experiments with the sartorius muscle of the frog (*Rana pipiens*) were conducted to obtain the data reported here. The maximum length of the intact muscle was carefully measured prior to excision and a measurement of the length was made when it was mounted in the holder to be certain that this latter length was greater than rest length in the animal. The excised muscle was mounted with its natural tendon attachments and a section of the attached bone at each end. A hole was drilled through each piece of bone and a small steel loop attached the bone directly to the experimental apparatus. The rationale of this experimental preparation will be discussed in detail in a later paper. When mounted, the sartorius muscle tension was adjusted to a static level of 0 7 g. The static tension was reset at this value whenever any change was made in the environment, such as a change in solution or temperature. The transducer was calibrated with a series of weights for each experiment.

For the isometric twitch tension studies, the muscle was stimulated once every two minutes with single pulses delivered by the mass electrodes. However, a tetanus stimulus was applied to the muscle early in each experiment, to make certain that all connections were tight and stable. In order to detect changes in the muscle, such as fatigue, during the course of an experiment, frequent checks were made on the amplitude of the twitch tension in response to current stimuli of chosen fixed amplitudes and durations. The muscle was allowed to equilibrate at each temperature for a period of at least ten minutes before measurements

were initiated. The current output of the stimulator was calibrated for each experiment.

The Ringer's solution contained, per litre, 6·7 g NaCl, 0·2 g KCl, 0·2 g anhydrous $CaCl_2$ and 0·1 g $NaH_2PO_4H_2O$. It was buffered to pH 7·2 with 0·15 M sodium phosphate. The curarized Ringer's consisted of a 1/50 000 w/v solution of tubocurarine chloride.

§ 3. Experiments and Results

3.1. *Variation of Stimulus Pulse Duration—Uncurarized Muscle*

It would appear that the possible significance of the effect of the duration of the single stimulus on the amplitude peak twitch tension *as a function of temperature* has been overlooked, since it is not uncommon to find no values listed for this parameter in much of the current literature (see table 1). Pertinent to the consideration of possible explanations for the previously indicated discrepancy between the results of Buchthal *et al.* (1957) and the classic results of Hill (1951) on the variation of isometric twitch tension with temperature, is the observation that Buchthal *et al.* employed a rather long duration stimulus pulse (5 msec) at *all temperatures*. Unfortunately, Hill (1951) does not indicate the duration of the pulses he employed, but it is not unreasonable to assume that the duration was short, possibly either 0·3 msec (Hill and Macpherson 1954) or in the general range of 1 msec (Hill 1953). In the present study, a detailed investigation was made of the effect on the amplitude of the isometric peak twitch tension of varying the stimulus pulse duration applied to uncurarized muscles in the temperature range 20° to 24·5°C. The current levels of the stimuli initially applied to each muscle were below the thresholds for eliciting detectable twitches. After a contraction of the muscle was detected, the stimulus current was increased in 0·01 amp steps at constant pulse duration with the muscle stimulated once every two minutes. This process was continued until a stimulus level was reached at which further increases in the current did not result in any increase in the amplitude of twitch tension. The amplitude of the stimulus current was increased beyond this point, but considerable care was taken that it was not increased to the point of causing injury to the muscle. This procedure was carried out on the same muscle at a number of stimulus pulse durations, starting with the commonly employed 0·2 msec followed by pulse durations of 1·0, 2·0, 3·0, 4·0, 5·0, 6·0, 7·0, 8·0, 9·0 and 10·0 msec.

The immediate result of this investigation was the finding that, for *all the stimulus pulse durations applied*, the amplitude of the twitch tension showed the usual rise in response to increasing current, followed by levelling off of developed tension; but, in general, for each increasing step in pulse duration (0·2 to 10·0 msec), the maximum amplitude of the observed tension was greater than that developed in response to shorter pulse durations. The major increases in peak tension are generally

Table 1. Uncurarized excised frog sartorii[a]—single responses[b]

Investigator	Pulse duration (msec)	Temperature (°C)	Peak tension (g)	$\frac{TL_0/M}{(kg/cm^2)/(g/cm^3)}$
Hill (1951), p. 349	Not given	21·9	Not given	0·415
Hill (1951), fig. 2D, lower row	Not given	21·5	14·7	0·39[c]
Hill and Macpherson (1954), fig. 7	0·3[d]	18·0	27·0	0·72[c]
Sandow (1944)	0·1	22–23	25·0 (av.)[e]	Not given
Prosser (1960) table I	Not given	21·9	7·5	Not given
Present paper	0·2	21·6	38·0	1·09
	2·0	21·6	66·5	1·90
	0·2	21·4	36·5	1·27
	2·0	21·4	56·5	1·96
	2·5	21·4	59·0	2·05
	4·0	21·4	61·0	2·12
	0·2	20·8	26·9	1·19
	1·0	20·8	32·6	1·46
	2·0	20·8	47·0	2·08
	3·0	20·8	50·6	2·24
	4·0	20·8	55·0	2·44
	10·0	20·8	59·0	2·63
	0·2	20·8	44·4	1·79
	2·0	20·8	53·3	2·15
	4·0	20·8	59·0	2·38
	10·0	20·8	61·4	2·48
	0·2	23·0	24·3	1·49
	10·0	23·0	45·9	2·81
	0·2	22·8	31·6	1·93
	10·0	22·8	52·1	3·19
	0·3	18·0	63·1	2·60
	2·0	18·0	80·0	3·30
	6·0	18·0	90·1	3·71
	10·0	18·0	95·0	3·91

Notes

a. Present authors' and Sandow's (1944) data obtained on *Rana pipiens*; remaining referenced authors do not indicate frog species.

b. Data grouped in each sequence of increasing stimulus pulse durations refers in each case to a single muscle.

c. Calculated from Hill's data.

d. Pulse duration assumed to be same as fig. 5 of the reference.

e. P. 225.

f. This muscle, on which observations were made at 18°C, is included here to compare with data given by Hill and Macpherson (1954) for the same temperature. The measured maximum capability for it lies at the upper end of the range of values observed by the authors for sartorii at high temperatures.

evident for pulse durations in the approximate range 0·2 to 6·0 msec, with usually only comparatively minor increases in tension for the range 6·0 to 10·0 msec.

Prior to presenting the quantitative experimental data, it is essential to consider the meaning of ' twitch response of the muscle '. In this paper this term does not refer to some specific twitch response of the excited muscle *in vivo*, but rather to responses elicited by stimulating excised muscle by mass electrodes, since data obtained on such preparations have constituted the basis on which previous investigators have drawn numerous conclusions regarding the contractile mechanism. A primary consideration is whether ' twitch response ' is to be defined solely in terms of the form of the contraction and relaxation phases of the myogram or whether an additional limitation is included in the definition, namely, that the response must be elicited by electrical stimuli of either a specific time duration (such as chronaxie) or by any time duration which is less than the spike potential interval.

With respect to the first possibility, such a definition might be reasonable for the purpose of comparing data if the variation in the value of the chronaxie with temperature were taken into account, and if it were consistently employed by previous investigators. However, since this is not the case, and since it appears to us that differences in the pulse durations employed by various investigators in order to study uncurarized skeletal muscle at high temperatures constitute one of the underlying reasons for the discrepant results reported on the variation of the amplitude of twitch tension with temperature, the primary aim of the investigation reported here was the achievement of responses to *single stimuli* which represent the *maximum capability* of the muscle under the particular environmental conditions. It is a direct observation from the experiments described in this paper that these responses of maximum capability are of the same form or shape as twitches of lesser amplitude. *A priori* there is no obvious simple relation between maximum capability levels in response to single stimuli and twitch levels limited by arbitrarily imposed bounds on the pulse duration. We feel that at this stage of understanding of muscle contractile mechanisms, it is important to identify a state of contractile tension which represents a ' limiting value ' for any specific choice of environmental parameters if the effects caused by varying these parameters are to be interpreted in terms of basic mechanisms.

With respect to the second possibility, namely, differentiating between responses elicited by ' supermaximal ' stimuli of pulse durations less than 3 msec (approximately equal to the spike duration at 20°c, Buchthal and Sten-Knudsen 1959) and those elicited by such stimuli of pulse durations greater than 3 msec, such a differentiation might well be of value in considering basic contractile mechanisms operating in muscles stimulated by mass electrodes. However, since the observed effects on the amplitude of tension are not confined to the application of stimulus

pulse durations longer than the interval of the spike potential but, in fact, are quite apparent for stimulus pulse durations in the range 0·2 to 3·0 msec, it would appear difficult to justify a restriction of this type at the present time, particularly since the excitation coupling mechanism is as yet unknown.

Fig. 1 exhibits typical isometric responses of a muscle at 24·5°c; (*a*) stimulated with a pulse of short duration (0·2 msec), and (*b*) stimulated with a pulse of long duration (10·0 msec). The amplitude of the developed tension in response to the longer stimulus pulse (fig. 1(*b*) is 2·6 times that of the tension developed in response to the shorter pulse (fig. 1 (*a*)) and the maximum time rate of tension development is 1·5 times faster for the response of fig. 1 (*b*) compared to that of fig. 1 (*a*). Both of the traces in fig. 1 represent responses that showed no further increase in tension

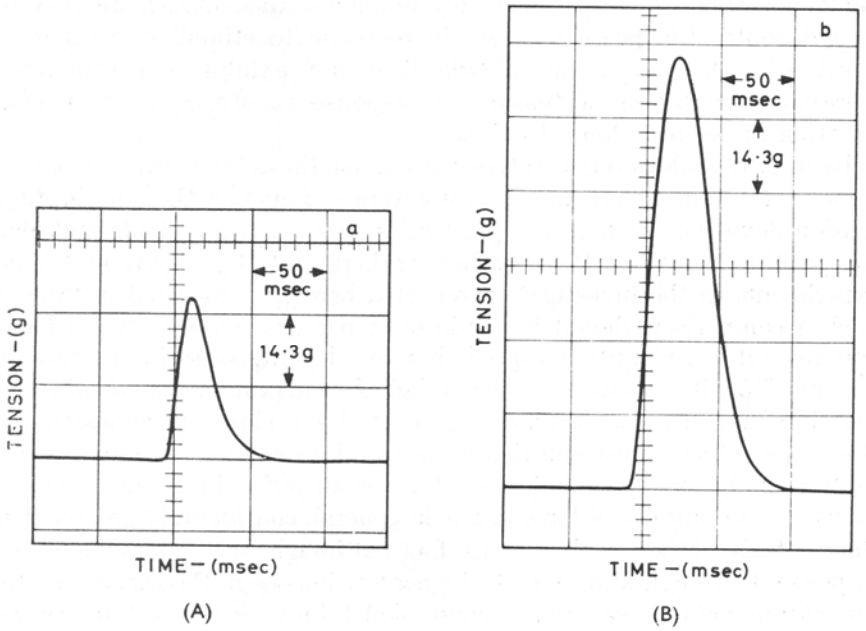

Fig. 1. Isometric responses of excised uncurarized frog sartorius muscle at 24·5°c, stimulated with ' supermaximal ' single stimuli delivered by a mass electrode—(*a*) and (*b*) represent responses of the same muscle for different stimulus pulse durations: (*a*) stim. dur. 0·2 msec, $TL_0/M=0{\cdot}94$; (*b*) stim. dur. 10·0 msec, $TL_0/M=2{\cdot}47$.

amplitude to increasing stimulus current at the pulse durations indicated. In other words, the criterion of ' supermaximal ' stimulation is applicable to both of the responses. It is obvious from the form of the recorded responses illustrated in the figure that the application of the longer duration pulse did not result in any distortion of the contraction or relaxation phases.

For the stimulus of long pulse duration (10 msec), one can consider whether the increased amplitude of observed tension may be the result of a second contractile process initiated at the termination of the pulse. That such is not the case is demonstrated as follows.† First, stimuli of very long duration, 20 to 30 msec, were employed in order to determine if a change in slope of the response, indicative of a second contractile process, could be detected. If the cut off of the pulse initiated a second response, then such a response could be easily detected if it occurred late in the rising phase of the first response, at the peak or thereafter. No evidence of such a response is apparent for any of the long duration stimuli. In contrast, when two stimulus pulses (0·2 msec pulse duration) separated by time intervals of 10 to 30 msec were applied to the same muscle, the second response was evident as a change in slope of the rising phase of tension. It should be noted here that prolonged pulse durations, 20 to 30 msec, were only employed to demonstrate that no second contractile process occurs in response to stimuli of long pulse durations. Of course, the muscle does not exhibit a continuously increasing amplitude of tension in response to stimuli of increasing duration for such prolonged pulses.

In order to make a quantitative comparison (in so far as this is possible) between the data of previous investigators and ours on the amplitude of tension developed at high temperature in response to a single stimulus, the published data of these earlier workers and the results of typical experiments in the investigation reported here are presented in table 1. Such a comparison should be made on a per unit cross-sectional basis, and since it is generally accepted that for the frog sartorius muscle the formula TL_0/M (T = twitch tension (kg); L_0 = maximum length of intact muscle (cm); M = muscle mass (g)) provides a close approximation of the output of the muscle on this basis, our data will be presented in that form. Study of table 1 indicates that for all pulse durations employed by us, the amplitudes of tension are, in general, considerably greater than those of the other authors. In fact, although the values listed are responses to a single stimulus, the higher tensions lie in the range reported for tetanus values measured on single skeletal muscle fibres (Ramsey and Street 1940, Casella 1951, Ramsey 1962). To our knowledge, these data represent the highest values reported for contractile responses to a single stimulus of frog skeletal muscle at the indicated temperatures. Specifically, it should be noted that in our experiments, pulse durations as short as 0·2 to 0·3 msec resulted in TL_0/M values ranging from 1·09 to 2·60 for temperatures in the range 18° to 23°c. Hill's (1951) and Hill and Macpherson's (1954) values range from 0·39 to 0·72 for the temperature range 18° to 21·9°c.

† Studies of the electrical response characteristics undertaken after completion of the investigations reported here indicate that no electrical response is elicited by the cut off of the stimulus pulse.

Fig. 2 shows a twitch tension curve (*a*) for a muscle at 21·5°C constructed from the data and trace presented in Hill's (1951) publication and, for comparison purposes, a typical tension response curve obtained by us (*b*) for a muscle at the same temperature (pulse duration 6·0 msec). The low amplitude and the slow time rate of rise of twitch tension of curve *a*, when compared with that of *b*, would appear to indicate that Hill's finding that the amplitude of twitch tension decreases with increasing temperature may have been associated with experimental conditions of measurement. However, Hill attributed observed low amplitudes of twitch tension at high temperatures to a presumed shortened duration of the active state. Our more complete data on the maximum time rate of rise of tension and its relation to stimulus pulse duration will be presented in a later paper. With regard to the data summarized in table 1, we suggest that the low tension outputs found by Hill, and Hill and Macpherson, and Sandow are primarily a result of inadequate stimulation of the muscle by a pulse of short duration.

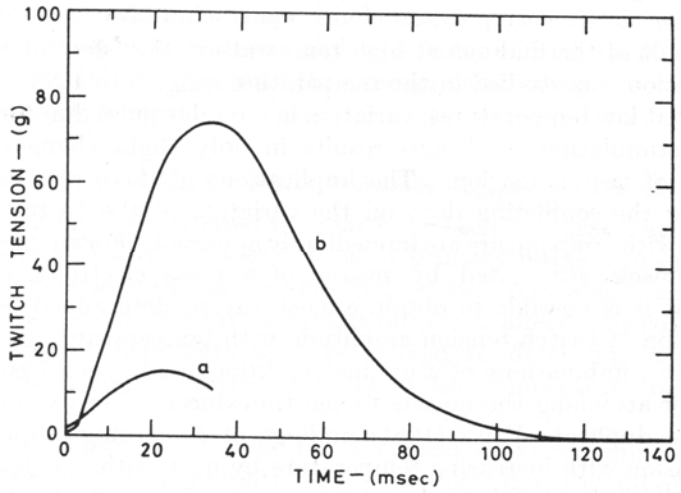

Fig. 2. Isometric response of excised, uncurarized frog sartorius muscle at 21·5°C elicited by a single 'supermaximal' stimulus: (*a*) reproduced from fig. 20, Hill (1951)—$TL_0/M = 0{\cdot}39$; (*b*) present authors—$TL_0/M = 2{\cdot}43$ (stim. dur. 6·0 msec).

Although, in general, the effect found for the variation of peak twitch tension with stimulus pulse duration is that indicated, we do not claim at the present stage of these investigations that each individual uncurarized muscle, at high temperature, will always respond with an increased amplitude of peak twitch tension to *each* increase of stimulus pulse duration over the entire range studied in the investigations reported here. The response of the uncurarized preparation at high temperatures to

different stimulating conditions evidently depends on the physiological state of the muscle. For example, in a few experiments no increase in the amplitude of peak twitch tension was found when the stimulus pulse duration varied between 0·2 msec and 2·0 msec. In one experiment at 22°C, the TL_0/M twitch value in response to a 0·2 msec pulse was 2·35 kg/cm^2/g/cm^3 with no additional increase in this value when the pulse duration was increased to 2·0 msec; however, a 19% increase occurred when the pulse duration was brought to 6·0 msec and only a 3% further increase occurred over this last value when the pulse duration was 10 msec. For an uncurarized preparation, one cannot rule out the possibility that with precise control of a number of factors such as fatigue of the muscle, geometric configuration of the stimulating electrical field, and other factors not yet recognized, the full twitch response at high temperature might be elicited with a comparatively short duration stimulus pulse. However, it is essential to realize that application of the presently accepted standard techniques for stimulating muscle can lead to grossly inadequate twitch responses for the uncurarized preparation.

As a result of the findings at high temperature, the effect of stimulus pulse duration was studied in the temperature range 0 to 1·5°C. It was found that at low temperatures, variation in stimulus pulse duration under optimum stimulating conditions results in only slight changes in the amplitude of twitch tension. The implications of these results on the reasons for the conflicting data on the variation of the twitch tension amplitude with temperature are immediately apparent. For an *uncurarized* skeletal muscle, stimulated by means of a mass electrode, we have shown that it is possible to obtain almost any predetermined result for the variation of twitch tension amplitude with temperature by choosing appropriate combinations of stimulus conditions and type of compliant element for attaching the muscle to the transducer. For example, it is possible to duplicate Hill's (1951) findings of decreasing amplitude of twitch tension with increasing temperature by using either a short duration stimulus pulse (of the order of 0·2 msec) and a particular length of compliant element, or by using a somewhat longer duration stimulus pulse (of the order of 1·0 msec) and a greater length of compliant element. Similarly, a constant stimulus pulse duration of the order of 2 msec, applied over the temperature range 0° to 35°C, when combined with a compliant element, resulted in a twitch tension, temperature relation with a minimum, as reported in a previous publication (Kelly and Fry 1958). Jewell and Wilkie (1958) indicated that they found both increases and decreases of twitch tension amplitude with increasing temperature, but in all cases the temperature coefficient was close to unity. Unfortunately, no information is given on the stimulus pulse durations employed or on the data obtained at high temperatures. We duplicated Jewell and Wilkie's results of a temperature coefficient close to unity by using a constant stimulus pulse duration of the order of 1·0 msec throughout the

temperature range studied and attaching the muscle to the transducer, as described in section 2. The duration of the constant stimulus pulse required to produce a particular effect, such as no variation of twitch tension amplitude with temperature, differs from muscle to muscle within a limited range of values. Jewell and Wilkie's finding, that the twitch tension amplitude increased with rising temperature in some experiments and decreased in others, presumably was due to the application of a stimulus pulse of the same duration in all experiments, at all temperatures. It is also of interest to note that during the course of the present series of experiments, it was observed that for stimulus pulse durations that result in maximum twitch tension the amplitude of the twitch tension increases with increasing temperature. This latter observation correlates well with the findings of Buchthal *et al.* (1957) on insect muscle. It is of considerable interest that in their work on locust muscle they used a 5 msec pulse duration stimulus at all temperatures.† It is also noteworthy that for a 20°C increase in temperature (10° to 30°C), Buchthal *et al.* found that the twitch tension amplitude increased by a factor of 1·56 for a single whole insect muscle, while we found for individual muscles increases as high as a factor of 1·5 for a 20°C change in temperature (1° to 21°C), with the average value being a factor of 1·4 for this temperature span.

3.2. *Variation of Stimulus Pulse Duration—Curarized Muscle*

All experiments discussed thus far have been concerned with uncurarized, normal excised muscles. It is of primary interest to determine if the phenomenon of increasing amplitude of twitch tension with increasing stimulus pulse duration (at high temperatures) is affected by curarization.‡ Accordingly, experiments were performed to determine the effect of pulse duration on curarized muscles in the temperature range 20° to 24°C.

Table 2 shows the results for a typical curarized muscle, namely, no increase in the peak amplitude of twitch tension as a result of increasing stimulus pulse duration. It should also be noted from table 2 that the maximum twitch tension output of the curarized muscle is considerably less than that of the same muscle, non-curarized and adequately stimulated. This difference in the amplitude of the contractile response between a curarized and uncurarized preparation would appear to be of fundamental significance and apparently is not simply associated with the

† Duration of spike for locust muscle is approximately 3·5 msec at 36°C (fig. 1, Neville and Weis-Fogh 1963).

‡ A concentration of 1/50 000 w/v of tubocurarine chloride was used. This concentration has been employed in a number of reported studies concerned with elucidating various aspects of the active state (Ritchie 1954, Lammers and Ritchie 1955, Ritchie and Wilkie 1955).

Table 2.

Preparation	Stimulus Pulse duration (msec)	Temperature (°C)	Peak tension (g)	TL_0/M (kg/cm²)/(g/cm³)
Uncurarized sartorius (single muscle)	0·2	20·8	39·6	1·62
	2·0	20·8	50·7	2·08
	6·0	20·8	58·7	2·40
	10·0	20·8	61·4	2·51
Curarized sartorius (single muscle)	0·2	20·8	32·8	1·32
	2·0	20·8	30·7	1·26
	6·0	20·8	28·0	1·14

blockage of the endplate transmission by the curare (as will be discussed in a later paper).

3.3. *Tetanus Tension Amplitude and Temperature Coefficient*

The muscle response of major interest in the investigations reported here is the isometric tension response to a single stimulus and, therefore, no *intensive* studies were made of tetanus tension. In eliciting the tetanus response, care was taken that at all temperatures the frequency of electrical stimulation was such that a smooth tetanus curve was obtained. At temperatures in the range of 0° to 4°C, a stimulus frequency of 20/sec was employed; in the range 8° to 12°C, 50/sec; and at 16°C and above, 100/sec. In general, the pulse duration employed for the tetanus stimuli was 1·0 msec. The stimulus frequencies applied at the various temperatures and the invariant pulse duration used were not based on the belief that these are the optimum conditions for maximum tetanus tension response. Rather, these were determined to be reasonable values to use until such time as a detailed investigation could be made to determine a stimulus frequency pattern or patterns to be applied to skeletal muscle to elicit optimum tetanus response. In view of this, and since at all temperatures the stimulation techniques and conditions were comparable with those of previous investigators, it was not expected that the observed results on tetanus tension would differ significantly from those previously reported in the literature. However, in the high temperature range (20° to 24°C) the observed amplitudes of tetanus tension were higher than any previously published for whole frog sartorius muscle. For example, in a series of experiments on 18 selected muscles in the temperature range 20° to 24°C, the TL_0/M value ranged from a minimum of 3·1 to a maximum of 4·8 (average 3·7). These values are in the same range as those reported for single fibres (Ramsey and Street

1940, Casella 1951, Ramsey 1962). Fig. 3 is a reproduction of an oscilloscope recording of a tetanus response of a muscle at 18°C. It seems

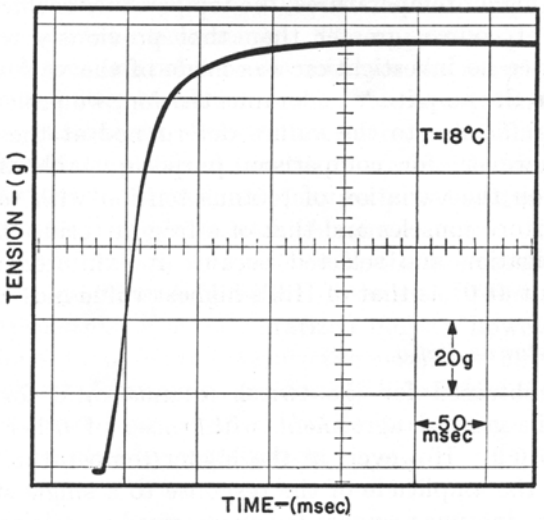

Fig. 3. Isometric tetanus response of excised, uncurarized frog sartorius muscle at 18°C, $TL_0/M = 4{\cdot}82$.

Table 3†.

Investigator	Temperature (°C)	Tetanus TL_0/M $(kg/cm^2)/(g/cm^3)$
Hill‡ (1951)	0	1·35
	7·1	1·63
	14·5	1·68
	21·9	1·87
Hill‡ (1951)	0	1·29
	12	1·64
	24	1·60
Hill‡ (1951)	0	2·20
	6·1	2·58
	11·8	2·70
	18·3	2·69
	25·1	2·78
Authors	0	2·28
	10·8	3·34
	21·9	3·82

† The individual groups of data refer to a single muscle in each case.
‡ Data on p. 349 of referenced paper, frog species not indicated.

reasonable to assume that the lower tension values obtained by previous investigators must have been associated with extraneous, compliant elements in the measuring system. In view of the greater tension outputs at the higher temperatures, the temperature coefficient of tetanus amplitude is, of course, greater than that previously reported in the literature. Since no investigation was made of the various factors that might influence the amplitude of tetanus tension, we prefer not to attach any special significance to the values determined at the different temperatures. However, for comparison purposes, table 3 shows Hill's (1951) results on the variation of tetanus tension with temperature for several frog sartorii muscles and that of a frog sartorius chosen from the present investigation and selected because it exhibited approximately the same output at 0° as that of Hill's highest value indicated in table 3.

3.4. *Twitch–Tetanus Ratio*

The values obtained for the twitch–tetanus ratio for temperatures near 0°C are in general agreement with those of other investigators, namely, 0·7 to 0·9. However, in the higher temperature range, values of the ratio of the amplitude of the response to a single stimulus to the amplitude of the tetanus response for uncurarized muscle are considerably different from those of previous investigators. For example, Hill (1951) indicates a twitch–tetanus ratio of 0·68 at 0°C and 0·22 at 21·9°C for frog sartorius muscle. In view of the results already presented in this paper, it can be readily appreciated that at the high temperatures the ratio of the twitch to tetanus is greatly influenced by the conditions of stimulation. Table 4 shows the result of a typical experiment conducted at approximately the same temperature as that of Hill (1951). In our experiment the various pulse durations were employed in the order indicated, and the maximum pulse duration was limited to 4·0 msec to illustrate that it is not essential to use pulses of the order of 10 msec to obtain high twitch–tetanus ratios. It should be noted that for this experiment there is a significant change in the twitch–tetanus ratio when the pulse duration is increased over the interval 0·2 to 2·0 msec.

Table 4.

Stimulus pulse duration (twitch) (msec)	Temperature (°C)	Twitch–tetanus ratio
0·2	21·4	0·43
2·0	21·4	0·66
2·5	21·4	0·69
4·0	21·4	0·72
2·0	21·4	0·66

All data refer to a single muscle.

It may be possible, by suitable manipulation of the pulse duration, to increase the amplitude of the twitch tension at high temperatures until the twitch–tetanus value is close to unity. However, we do not believe that such a result, *per se*, will contribute to any deeper understanding of basic muscle mechanisms. The important point is that the amplitude of both tensions must be maximal for the muscle for each type of stimulus. It is quite possible to achieve twitch–tetanus ratios close to unity, because the output of the tetanus tension is below its optimum level, and as already emphasized here in reviewing the results of earlier investigators, the twitch–tetanus ratio may be quite low due to inadequate eliciting of twitch tension output at high temperatures. In the present investigation, for the uncurarized muscle in the temperature range 20° to 24°c, values of 0·7 to 0·8 were obtained for the ratio of the single response to the tetanus response. For the curarized muscle, however, the ratios found were of the order of 0·3 to 0·4 since the maximum twitch amplitude exhibited by a curarized muscle is considerably below that of an adequately stimulated, uncurarized preparation, while the tetanus amplitude is comparatively unaffected by curarization.

§ 4. Discussion

As indicated in the introduction, the discussion of the results of this paper will be included in a subsequent paper.

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APPENDIX

The paper indicates that the relation between the 'supermaximal' amplitude of tension developed by frog skeletal muscle in response to a single stimulus at high temperature is a monotonically increasing function of the pulse duration. One possible explanation of the observed increase of tension with increased pulse duration is that long duration stimuli cause multiple action potentials in the muscle. It is of interest in this regard to mention briefly our preliminary results on the electrical response characteristics of frog sartorius muscle stimulated with pulses of various durations in the range 0·2 to 10·0 msec.

The experimental procedures for the electrical response studies were essentially that described above, except for the additional arrangements required for measuring action potentials. The muscle was stimulated by a mass electrode and the electrical responses were picked up by gross electrodes placed at the pelvic end of the muscle.

Although the results will not be described in detail here, certain pertinent data can be indicated. First, the increased tensions elicited by the long duration pulses are not the result of prolonged repetitive discharges not present when the muscle is stimulated by short duration stimuli. The number of action potentials recorded on stimulation of a muscle by a single 10·0 msec pulse is no different than the number recorded in response to a 0·2 msec duration pulse. In general, if precautions to avoid the use of excessive levels are observed in regard to stimulating currents applied to the muscle, then two distinct electrical responses are recorded from the uncurarized muscle for all pulse durations employed. If evidence conceivably interpretable as support for additional small action potentials is recorded, such is not restricted to occasions of stimulation by long duration pulses but is equally evident when the muscle is stimulated by short duration stimuli.

Summary

An attempt was made to resolve experimentally the conflicting data on the variation of the amplitude of isometric twitch tension with temperature for frog skeletal muscle. It was shown that the use of mass electrodes, to provide ' supermaximal ' stimuli for excised, uncurarized frog sartorius muscle can lead to low values of ' peak ' twitch tension at temperatures above 18°c, if the stimulating current amplitude is the only variable parameter. In order to determine the peak tension, two parameters, amplitude and duration, must be varied. The application of different durations of ' supermaximal ' stimuli, and the use of widely differing types of mechanical attachments between muscle and transducers by previous investigators, may account for the conflicting results.

The effect of pulse duration of a single stimulus on the amplitude of elicited tension was found to be temperature dependent, with a minimal effect at 0°c. The maximum amplitude of isometric tension in response to a single stimulus for uncurarized frog sartorius muscle was shown to increase with increasing temperature. The maximum amplitudes of isometric tetanus tension at temperatures above 18°c are considerably greater than those reported by previous investigators. This is attributed primarily to improved measurement methods, in particular to arrangements which prevent loss of tension due to the compliance of attachments between muscle and transducer.

Values of the ratio of the twitch to tetanus tension as high as 0·8 have been obtained, as compared with the generally reported range of values 0·1 to 0·3.

The maximum amplitude of tension developed by a *curarized* muscle in chloride Ringer's, at temperatures above 18°c, in response to a single pulse delivered by a mass electrode is considerably less than that of an uncurarized muscle adequately stimulated at the same temperature. Increasing the duration of the stimulus does not overcome the barrier of the curarization.

Résumé

On a essayé de résoudre expérimentalement les données contradictoires sur la variation d'amplitude de la tension isométrique de la secousse dans le muscle strié d'une grenouille avec température. On a prouvé que l'emploi des électrodes " de masse " pour obtenir des stimulus " super-maximaux " pour le muscle couturier excisé non-curarisé d'une grenouille peut conduire à de basses valeurs de la tension " de crête " de la secousse aux températures au-dessus de 18°c, si le seul paramètre variable est l'amplitude du courant stimulateur. Afin de déterminer la tension de crête il est nécessaire de varier deux paramètres, à savoir l'amplitude et la durée. Les résultats contradictoires peuvent être expliqués par l'application de diverses durées de stimulus " super-maximaux " et par l'emploi de très différents types de joints mécaniques entre le muscle et les transducteurs dans les recherches d'investigateurs antérieurs.

On a trouvé que l'effet de la durée d'impulsion d'un stimulus unique sur l'amplitude de la tension produite dépend de température, l'effet minimum ayant lieu à 0°c. On a trouvé de même que l'amplitude maxima de la tension isométrique produite par un stimulus unique dans le muscle couturier non-curarisé d'une grenouille augmente lorsque la température s'élève. Les amplitudes maximum de la tension isométrique de tétanos au-dessus de 18°c sont bien plus grandes que celles trouvées par les auteurs antérieurs. Ceci est attribué en premier lieu au perfectionnement de méthodes de mesure, en particulier aux dispositifs qui empêchent la perte de tension, causée par la flexibilité des joints entre le muscle et le transducteur.

Pour le rapport entre la tension de secousse et celle de tétanos on a obtenu des valeurs s'élèvant jusqu'à 0,8, tandis que les valeurs connues généralement sont de 0,1 à 0,3.

L'amplitude maxima de tension développée par un muscle *curarisé* dans la solution (chlorure) de Ringer aux températures au-dessus de 18°c par une impulsion unique venant d'une électrode " de masse " est bien plus petite que celle d'un muscle non-curarisé, stimulé convenablement à la même température. L'augmentation de la durée du stimulus ne surmonte pas la barrière de curarisation.

Zusammenfassung

Es wurde versucht, die einander entgegengesetzten Daten betr. die die Amplitudenänderung der isometrischen Zuckungsspannung begleitende Temperatur änderung für den Frosch-Skelettmuskel auf experimentellem Wege zu lösen. Es wurde gezeigt, dass die Anwendung von Masselektroden zur Erzeugung der " supermaximalen " Stimuli für einen ausgeschnittenen nichtkurarisierten Schneidermuskel des Frosches zu niedrigen " Spitzen "-Zuckungsspannungswerten bei Temperaturen oberhalb 18°c führen kann, falls die Amplitude des stimulierenden Stromes der einzige veränderliche Parameter ist. Um die Spitzenspannung zu bestimmen, muss man zwei Parameter, Amplitude und Dauer, ändern. Die entgegengesetzten Resultate können durch die verschiedentliche Dauer der " supermaximalen " Stimuli, sowie dadurch, dass die früheren Forscher äusserst verschiedene Typen von mechanischen Befestigungen zwischen dem Muskel und den Wandlern anwandten, erklärt werden.

Es wurde gefunden, dass die Wirkung der Impulsdauer eines Einzelstimulus auf die Amplitude der erzeugten Spannung temperaturabhängig ist, wobei der Minimaleffekt bei 0°c liegt. Es wurde gezeigt, dass die Maximalamplitude der isometrischen Spannung, welche erzeugt wird, wenn ein nichtkurarisierter Schneidermuskel des Frosches einen Einzelstimulus erhält, mit steigender Temperatur ansteigt. Die Maximalamplituden der isometrischen Tetanusspannung bei Temperaturen oberhalb 18°c sind erheblich grösser als die von den früheren Forschern angegebenen. Dies ist hauptsächlich den verbesserten Messverfahren zugeschrieben, insbesondere den Einrichtungen, die den durch die Nachgiebigkeit der Zwischenstücke zwischen Muskel und Wandler verursachten Spannungsverlust verhindern.

Die erhaltenen Werte des Zuckungs/Tetanus-Spannungsverhältnisses belaufen sich auf bis 0,8, wobei die üblich angegebenen Werte von 0,1 bis 0,3 ausmachen.

Die Maximalamplitude der bei Temperaturen oberhalb 18°c in einem *kurarisierten* Muskel in Ringer's Chloridlösung erzeugten Spannung, die durch einen von einer Massenelektrode gelieferten Einzelimpuls verursacht wird, ist beträchtlich kleiner als die eines bei derselben Temperatur entsprechend gereizten nichtkurarisierten Muskels. Die Verlängerung der Reizdauer überwindet den hinlänglich Kurarisationswall nicht.

Резюме

Сделана попытка экспериментального решения вопроса противоречивых данных по изменению амплитуды изометрического напряжения судороги с температурой для скелетной мышцы лягушки. Показано, что применение массовых или многократных электродов для подачи «сверхмаксимальных» стимулов вырезанной, некураризованной портняжной мышце лягушки может быть причиной низких значений «пикового» напряжения судороги при температурах выше 18°C, если единственным переменным параметром является амплитуда стимулирующего тока. Для определения пикового напряжения необходимо варьировать два параметра: амплитуду и продолжительность. Противоречивые результаты могут быть обьяснены применением различных

периодов продолжительности «сверхмаксимальных» стимулов и тем, что исследователи употребляли до сих пор самые разнообразные типы механических закреплений между мышцей и трансдукторами.

Оказалось, что эффект продолжительности импульса единичного стимула на амплитуду вызванного им напряжения зависит от температуры, причем минимальный эффект происходит при 0°C. Было найдено, что максимальная амплитуда изометрического напряжения, вызванного единичным стимулом в некураризованной портняжной мышце лягушки, увеличивается вместе с температурой. Максимальные амплитуды изометрического напряжения столбняка при температурах выше 18°C гораздо больше данных, находящихся в литературе по этому предмету. Это можно объяснить главным образом улучшением измерительных методов, в особенности устройствами, которые предотвращают потерю напряжения, вызванную податливостью закреплений между мышцей и трансдуктором.

Полученные значения отношения напряжения судороги к напряжению столбняка достигают 0,8, в то время как обычно приводимые значения колеблются от 0,1 до 0,3.

Максимальная амплитуда напряжения *кураризованной* мышцы в хлоридном растворе Рингера при температурах выше 18°C, вызванного единичным импульсом от массового электрода, значительно меньше амплитуды некураризованной мышцы, стимулированной соответственно при той же температуре. Увеличение продолжительности стимула не преодолевает барьера кураризации.

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