THE EFFECT OF TEMPERATURE ON LOCUST FLIGHT MUSCLE

By A. C. NEVILLE and TORKEL WEIS-FOGH

*Zoophysiological Laboratory B, 36 Juliane Maries Vej,
Copenhagen University, Denmark*

In a recent study of the motor activity in relation to the wing movements of flying desert locusts, muscle action potentials were used to indicate mechanical activity of single motor units and also to demonstrate the motor repertoire of the central nervous system at the unit level (Wilson & Weis-Fogh, 1962). In order to do this it was essential to know exactly how the recorded potentials are related to the mechanical response at the relatively high thoracic temperature of flying locusts, 30–42° C.; this information is provided here. During flight at medium intensity most muscles are fired a single time per stroke cycle and the movements are based upon ordinary twitch activity, but in some control reactions and during more powerful flight, D. M. Wilson discovered that locusts often make use of double firing, i.e. a unit is fired by two *closely spaced* action potentials (in the locust normally 4–8 msec.). In cases where the tetanus:twitch ratio is higher than unity this can obviously result in more force and power and he suggested that this may provide an important control mechanism in such systems which operate essentially on a twitch basis (Wilson, 1960). It was therefore of interest to investigate the mechanical effect of double firing.

In common with many other orders of insects, locusts move their wings by ordinary striated muscle in which there is the usual one-to-one relationship between nerve impulse and twitch contraction (Roeder, 1951). Although these muscles do not reach as high frequencies as the myogenic fibrillar muscles (Pringle, 1949), they may, nevertheless, vibrate the wings at a rate of 100 complete wingstrokes per second (Sotavalta, 1947). It is more economical if, instead of working against each other, the two antagonistic sets of wing muscle (elevators and depressors) apply their work mainly to move the wings. This requires that the mechanical change in a twitch be almost completed within the period of the relevant half cycle of the wingstroke. However, even in the relatively slow desert locust the twitch durations measured earlier are somewhat large to comply with this requirement (Buchthal, Weis-Fogh & Rosenfalck, 1957), probably because it was difficult to keep the muscle in a healthy state at high temperature. Since it is now known that double firing occurs as a normal event and will increase the duration of a contraction, it was obviously desirable to re-investigate the problem.

The technique was designed so as to make the results obtained with the isolated muscle directly applicable to studies of the intact animal.

MATERIAL AND METHODS

The large dorsal longitudinal depressor of the hindwings of cage-bred *Schistocerca gregaria* Forskål (*phasis gregaria*) was used throughout since there is no indication that this muscle differs significantly from other major wing muscles with respect to the properties investigated here (Ewer & Ripley, 1953). It is innervated by a nerve, 8–9 mm. long, with five large motor axons, one for each of the topographically distinct motor units (Neville, 1963). Each fibre is innervated at several points along its length so that membrane depolarization occurs almost simultaneously in the entire muscle and gives rise to non-facilitating and probably non-propagated action potentials of the fast type (Hagiwara & Watanabe, 1954). It is a typical twitch muscle (Buchthal *et al.* 1957) which sustains tetanic contractions badly and requires a constant supply of oxygen but whose contractile properties are essentially like those of other striated muscle (Weis-Fogh, 1956*a*).

The pterothorax of recently matured adult females was isolated, sagittally bisected and freed from remains of gut and central nervous system, mounted horizontally by means of adhesive wax to a solid support, immersed in a constant-temperature bath with saline and perfused with air, as described earlier (Weis-Fogh, 1956*a*). The dorsal longitudinal muscle in the segment in front (mesothorax) was removed in order to free the anterior insertion of the metathoracic muscle (the second phragma), the other end being connected to the support via thick solid cuticle. In this way the resting muscle remained slightly extended by the spring action of the notum which, however, is much more compliant than the passive-elastic part of the muscle itself. The isolated two-pronged tip of a steel cantilever was mounted vertically on a Prior micromanipulator and pushed down along the phragma from the medial side; the muscle was extended and both resting and active tension were measured by means of a RCA 5734 mechano-electrical transducer connected with the cantilever (resonance frequency 300 cyc./sec.) The stiffness of this linear spring was such (0·0083 mm./g.) that an average muscle shortened auxotonically between 3 and 4 % of its length, i.e. close to the optimum shortening for work production in isotonic twitches (Buchthal *et al.* 1957).

In order to ensure recording conditions similar to those in the flying animal, stimulation was effected through the nerve and was always adequate to fire the five motor axons simultaneously (0·05 msec. duration, hooked double electrodes, insulated after application by petroleum jelly, DISA multistimulator). The muscle potentials were picked up by an external monopolar platinum-iridium electrode (0·1 mm.) insulated to the tip and inserted superficially about 2 mm. from the entrance of the nerve where the almost synchronous firing of the units was recorded as a compound, almost monopolar, negative spike corresponding to 2–6 mV. at the muscle membrane (Fig. 1), i.e. very similar to recordings from the flying animal (Wilson & Weis-Fogh, 1962). The neutral electrode was a ring of uninsulated platinum in the bath. A Tektronix type 122 pre-amplifier and type 502 dual-beam oscilloscope served for recordings.

RESULTS

In auxotonic contractions against a linear spring, as in the present experiments, the active force is proportional to the shortening and the work done on the spring during shortening or the work done by the spring on the muscle during lengthening (relaxation) is then related to the second power of the relevant displacements. In the records of mechanical change this means that *small* deviations from the base line (which represents resting tension) are of little energetic significance. Since the tension appeared to decay exponentially under all conditions, as in frog muscle (Jewell &
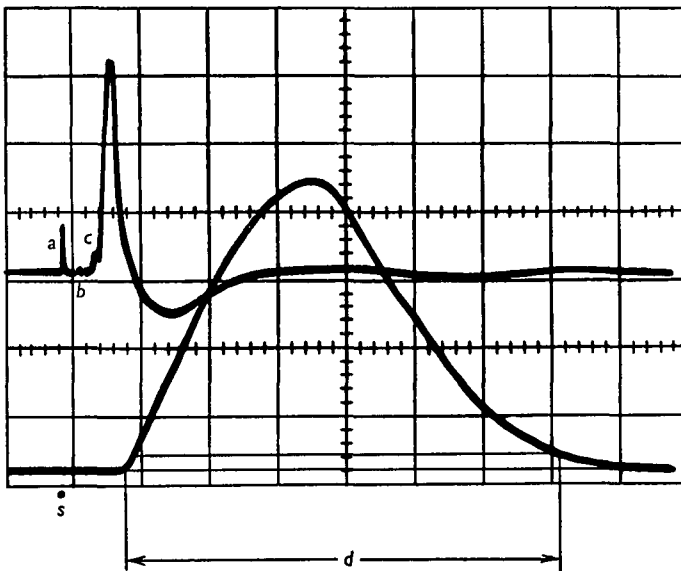

Fig. 1. Muscle action potential (upper trace) and auxotonic twitch force (lower trace) of locust flight muscle at 36° C. (*a*) stimulus artifact, also indicated directly by dot (*s*) on lower tracing: (*b*) nerve activity: (*c*) indication of slight asynchrony between motor units: (*d*) total duration, as defined in the text. Sweep speed 5 msec./cm. grid; voltage 2 mV./cm. grid at muscle surface; force 8 g./cm. grid. Total twitch work 0.66 g.cm.

Wilkie, 1960), the total duration of a twitch or of a doubly fired contraction was therefore defined as the time from the first sign of tension rise and until 95 % of the active tension had disappeared. This means that with resting tensions 0.2 to 0.25 of the active twitch tension, less than 2 % of the total work remained in the spring when the twitch was said to be over. As can easily be worked out, the ratio R between the work at a given tension and the total work in a contraction is $R = (c_1 c_2 + 0.5 c_2^2)/(c_1 + 0.5)$, where c_1 = resting tension/maximum active tension and c_2 = given tension/maximum active tension.

Twitch. Fig. 1 shows the typical relationship between mechanical and electrical changes at 36° C., the base-line representing 8 g. of resting tension. Between the stimulation artifact (*a*) and the large action potential of the muscle are ripples (*b*) caused by activity in the five motor axons. The small hump (*c*) on the rising part of the action potential indicates some asynchrony between the units but since it is very small and since it is known that all 5 motor units follow the same contraction course (Neville,

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1963), the durations of the mechanical change in Fig. 2 can be considered representative for the *whole* muscle. As in the earlier experiments, the duration decreases by a factor of 1·5 from 30 to 40° C., but the absolute values here are about 30 % smaller than previously found (Buchthal *et al.* 1957). A few representative figures are given in Table 1. It is seen that the muscle is somewhat faster than the fastest mammalian muscle known, the inferior oblique eye muscle of the cat, in which the rise time is 19 msec. (Wills, 1942) against 15 msec. in the flight muscle, measured at the same temperature.
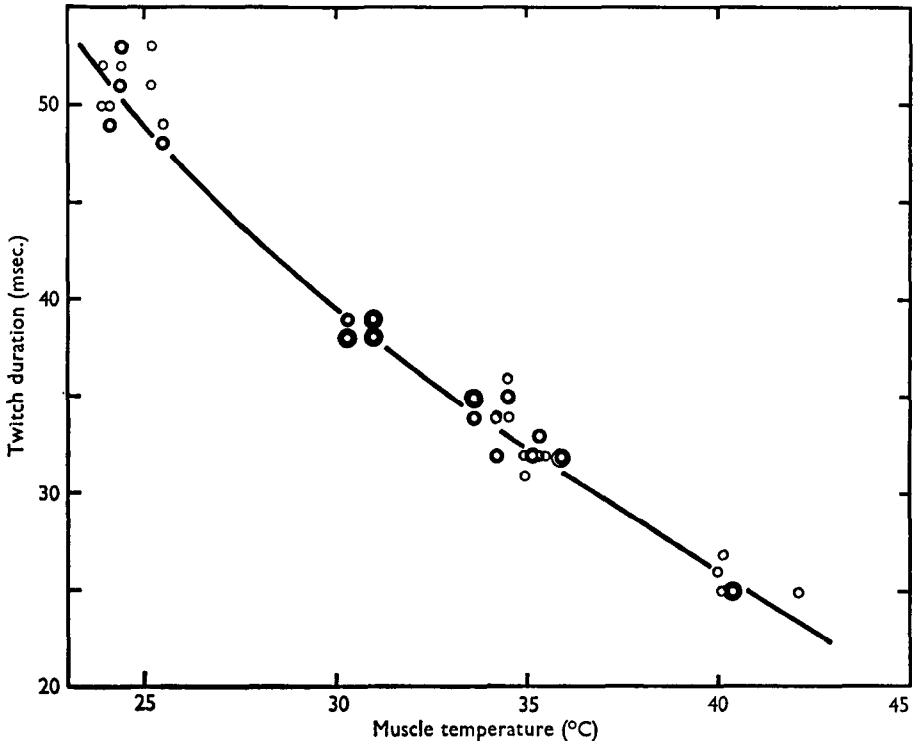

Fig. 2. Total duration of single twitches of locust flight muscle. Results from nine muscles most of which were tested at two or three temperatures.

As to the time relationship between action potential and contraction, the results from nine muscles are seen in Fig. 3. In curves (*A*) and (*B*) the time begins with the stimulation of the nerve (0·05 msec. duration) and includes nerve propagation (1·1 msec. at 36° C.; 8 m./sec.) as well as the neuromuscular delay (about 1·5 msec. at 36° C.) both of which are known to be temperature-dependent. Since the nerve was stimulated close to its emergence from the ganglion, the results in Fig. 3*A* show that at 36° C. the motor neurons of the flying animal must fire 4–5 msec. earlier than the time at which the muscle action is required, i.e. an appreciable fraction of a typical wingstroke period which is 50–60 msec. This must be taken into account in studies of the nervous control of flight.

Fig. 3*C* is interesting for two reasons. First, it shows that the delay between the well-defined peak of the action potential and the onset of the mechanical change is

almost independent of temperature throughout the flight range and amounts to only 1 msec. This means that similar monopolar recordings of action potentials in flying locusts can be interpreted accurately with respect to the beginning of contraction of a
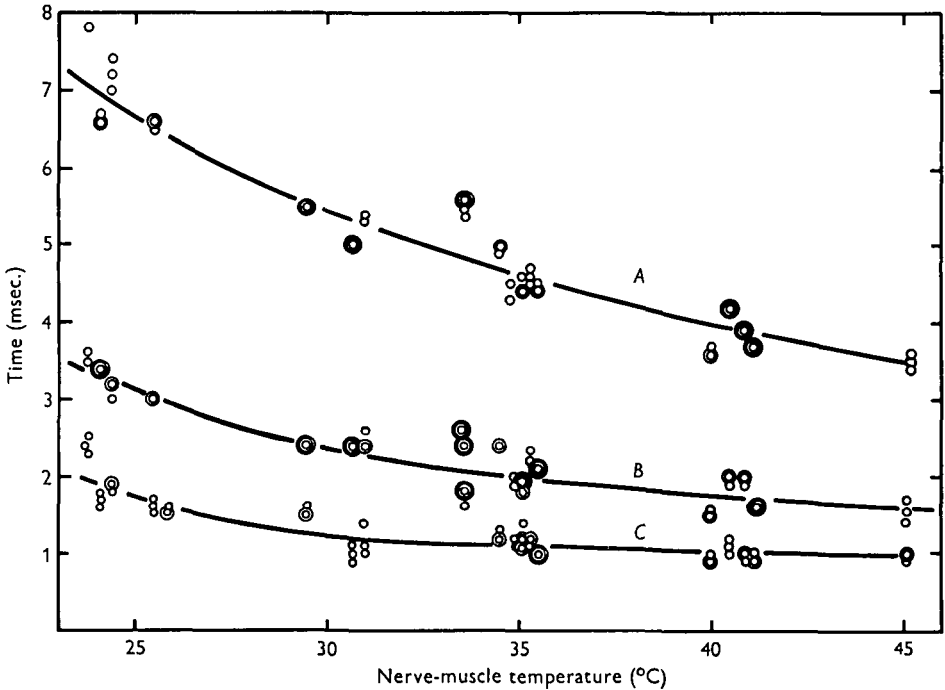

Fig. 3. Some time relationships between muscle action potential and mechanical change in locust flight muscle. (*A*) delay from nerve stimulation near ganglion to onset of twitch. (*B*) delay from nerve stimulation to earliest sign of electrical change of muscle membrane. (*C*) delay from the peak of the action potential to the onset of the twitch.
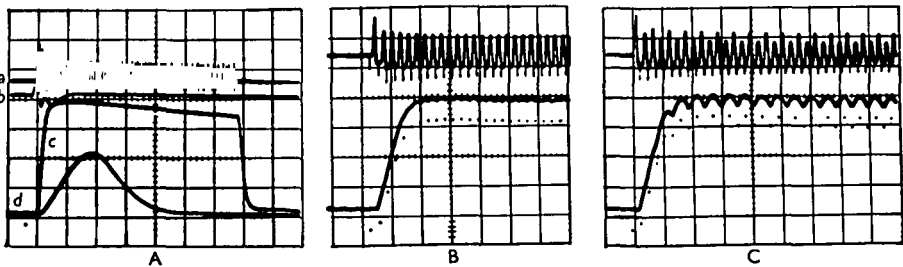

Fig. 4. Tetanic contractions with ripples. (A) comparison between twitch at 32° C. (*b* and *d*, sweep speed 10 msec./grid unit) and tetanus (*a* and *c*, sweep speed 100 msec./grid unit); stimulation frequency 114 cyc./sec. (B) tetanus with ripples at 35° C.; stimulation and response frequency 180 cyc./sec. (sweep speed 20 msec./grid unit). (C) same as (B) but stimulation intensity slightly decreased with rhythmical dropping out of motor units as a result; note the relation of two action potentials to one visible ripple.

given unit although the thoracic temperature may remain unknown (Wilson & Weis-Fogh, 1962). Secondly, if one admits that the quick initial change in potential (rise time about 1 msec.) represents the first link in the transmission of excitation from the muscle membrane to the contractile fibrils, the remaining links can hardly involve any

time-consuming chemical reactions but probably depend upon physical processes, because the delay remains almost constant from 30 to 45° C.

Tetanus. At low temperature (3° C.) the tetanus:twitch ratio approaches unity, rising to 1·3 at 11° C. and reaching 2·0 at 25° C. (Buchthal *et al.* 1957) but between 25 and 42° C. we found that the ratio was independent of temperature and averaged 2·0 (Fig. 4A; range in eight muscles from 1·72 to 2·38) and there was no systematic change in tetanic force. In other words, twitch work and force do not depend on temperature in the flying animal and it is possible that double firing may result in considerably more work than is produced in a twitch.

During tetanic contractions some fatigue was always observed and, in contrast to trains of single twitches (cf. Fig. 7A), a small residual extra tension remained for a considerable time after the major part of the relaxation had taken place at approximately the same rate as in a single twitch (Fig. 4A). The high degree of synchrony
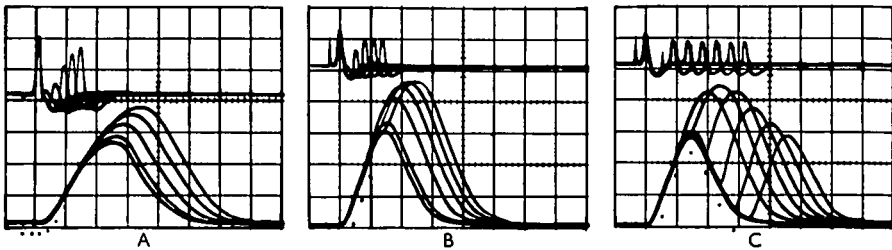

Fig. 5. The effect of double firing shown on superimposed photographs of electrical changes (upper traces) and mechanical force (lower traces) when the delay between the first and second firing is gradually increased. (A) flight muscle at 24° C. (B) another muscle at 35° C. and (C) the same muscle with longer delays. Sweep speed 10 msec./cm. grid.

between the motor units and the probable absence of a plateau of full activity both become apparent in Fig. 4B in which the small ripples on the mechanical record are seen to be uniquely related to the action potentials at 180 stimuli per second. When the intensity of stimulation was slightly decreased, as in Fig. 4C, one or two units dropped out by rhythmically missing beats due to the refractoriness of the nerve axons, in accordance with the observations of Ewer & Ripley (1953), the effect being abolished when the intensity was at least twice the threshold for a single twitch. In any case, it is clear that the nerve and muscle membranes do not represent any potential barrier to high wingstroke frequencies, a result which was corroborated by experiments with double firing.

Effect of double firing. In flying locusts it was found that both multi-unit and single-unit muscles sometimes fire doubly and the second muscle potential is then smaller than the first by an amount depending on the time separation between the two (Wilson & Weis-Fogh, 1962). The superimposed oscilloscope sweeps in Fig. 5 offer examples of how the second action potential of the isolated muscle increases in size with increasing duration between the first and second firing at two representative temperatures. It is seen from Fig. 5B that at 35° C. a separation as small as 2 msec. is discernible in both electrical and mechanical records. Under these conditions the contraction resembles a smooth twitch. Even a small increase of the interval to 4 or 5 msec. resulted in 30 % more force, or 50–60 % more work, and one could see a small

increase in shortening velocity indicating that the plateau of full activity lasts less than 4 msec. at this temperature (cf. Macpherson & Wilkie, 1954) if, in fact, such a plateau exists at all in this muscle. At longer intervals a maximum is reached beyond which the force declines again and the two contractions become clearly separated. When the interval corresponds to the twitch duration as here defined, there is no mechanical summation left (Fig. 5 C) and twitches can follow each other in trains with no summation and no change in amplitude, i.e. there is no staircase effect, as is sometimes found
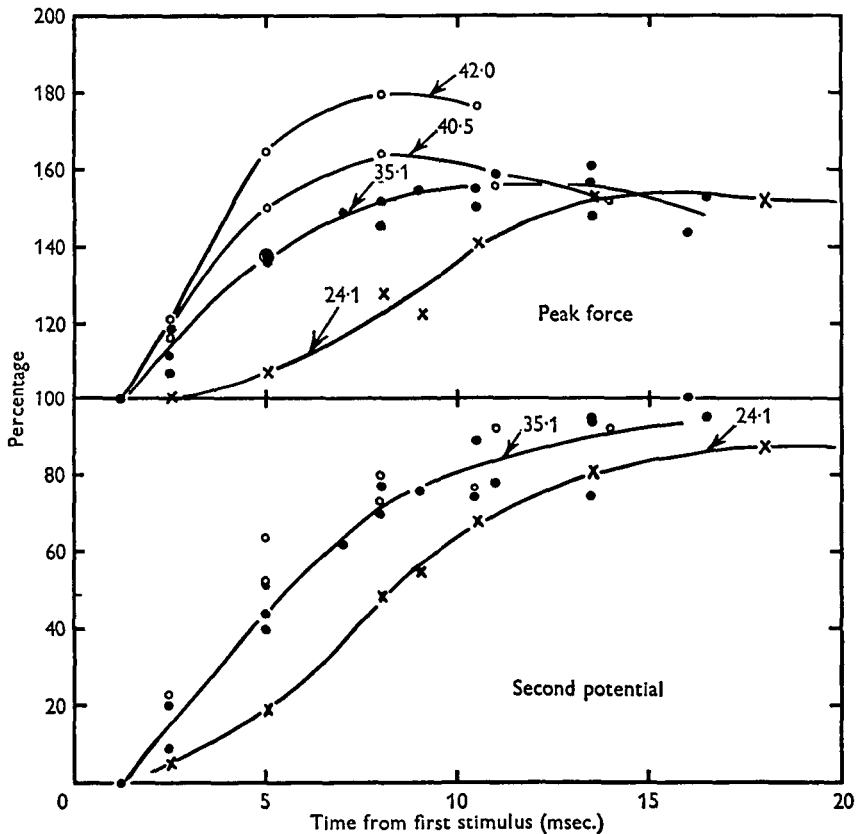

Fig. 6. The effect of double firing on peak force (in percentage of the force in single twitches; upper curves) and on spike size of the second action potential (in percentage of the first; lower curves). The abscissa is the delay in stimulation between the two firings. The temperature is indicated.

in frog muscle (cf. Fig. 7). In some experiments it was found that a twitch following immediately after a short tetanus had increased amplitude, corresponding to the so-called post-tetanic potentiation (cf. Ritchie & Wilkie, 1955), but it was absent in the best aerated muscles and in these preparations it was also found that the total duration of doubly fired contractions equalled only the twitch duration plus the time interval between the two stimuli. (In some early experiments the duration had increased by an extra 3–10 msec.). It is therefore concluded that in *trains* of both twitches and doubly fired contractions there is no interaction between the single events other than the mechanical summation seen in Fig. 5.

As to quantities, Fig. 6 shows that double firing results in as much as 50–80 %
extra force, i.e. 100–175 % *extra* work, and that it is possible to obtain a rough esti-
mate of the work increase from the size of the second action potential relative to the
first.

<div align="center">DISCUSSION</div>

Fusion of contractions. The absence of both staircase effect and post-tetanic potentia-
tion is in accordance with the finding that, with identical results, we could estimate the
time interval at which summation begins in three independent ways: (1) indirectly
from the duration of single twitches (Fig. 2), (2) from experiments with double
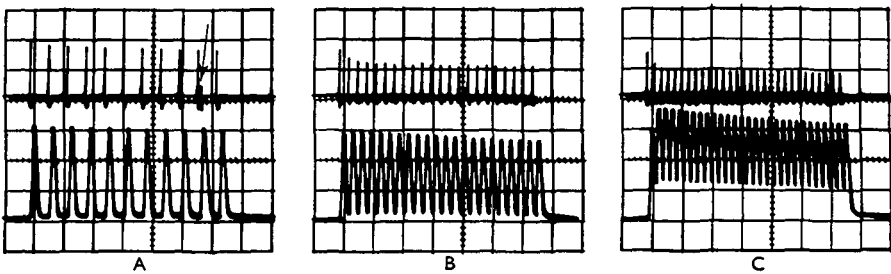firing such as those in Fig. 5C, and (3) from recordings of trains of twitches as in

Fig. 7. Trains of singly fired twitches at 34° C. with repeat intervals 62·7 msec. (A), 31·2 msec.
(B), and 23·2 msec. (C), respectively. The normal wing-stroke period of an animal of this size
is 59 msec. The arrow in (A) shows a spontaneous double firing.

Fig. 7. In the latter example, the normal wingstroke period of the locust in question
was 59 msec. and at 34° C. there was no fusion whatever at repeat intervals of 63 msec.
(A), whereas the base line was raised 10 % at 31 msec. (B, corresponding to about 4 %
of work) and by 30 % at 25 msec. intervals (C, about 16 % of work). In other words,
two antagonistic twitch muscles could operate a wing at the normal wingstroke fre-
quency without any significant waste of work due to mutual interaction. In the case
of doubly fired contractions with an interval between first and second firing approaching
the optimum for work production, about one tenth of the energy would be wasted.

Flight and temperature. In the absence of radiant heat, a desert locust is able to
start flight at an air temperature of 22° C. but it cannot maintain flight below 24–25° C.;
it is also characteristic that the flight is independent of ambient temperatures from 25
to 35° C. and that it begins to deteriorate above 37° C. (Weis-Fogh, 1956b). These
observations are explicable from the results summarized in Table 1. The 'waste' of
mechanical work due to mutual interaction of antagonistic muscles was calculated
according to the formula on page 113. These *approximate* estimates presuppose (1) that
the resting tension is caused by a simple passive-elastic component in the muscle
(Buchthal *et al.* 1957), (2) that the downstroke lasts 60 % of the entire wingstroke
cycle, i.e. 35 msec. in a female of average size (Weis-Fogh, 1956b), and (3) that
stretching of the incompletely relaxed dorsal-longitudinal muscle requires the same
amount of work as is represented by the deviation from the base-line at the time when
the antagonist begins to contract. The experimental justification for the latter assump-
tion is meagre since the only published measurements of the stretching of a muscle

at a late stage of relaxation seem to be Fig. 2 in Abbott, Aubert & Hill (1951). Their results are in accordance with the above procedure especially when it is remembered that the stretching will be relatively slow in the flying animal. Actually, due to the momentum of the wing, the muscle is released to some extent before it is stretched by the antagonist so that the figures in Table 1 represent conservative estimates. At present, it is not known whether the upstroke muscles are faster than the downstroke muscles, corresponding to the difference in duration of the two half cycles.

Table 1. *The influence of temperature on the total duration of contractions of the dorsal-longitudinal flight muscle, together with approximate estimates of the amount of work which the antagonistic upstroke muscles must spend in order to extend the incompletely relaxed downstroke muscles ('work wasted', in percentage of mechanical work done)*

(a) During train of single twitches; (b) during train of doubly fired contractions each giving 30 % more force and 50–60 % more work than a single twitch, and (c) when each gives 50–80 % more force and 100–175 % more work than a single twitch.

	(a) Single twitch		(b) Doubly fired contraction (intermediate output)		(c) Doubly fired contraction (maximum output)	
Muscle temperature	Duration (msec.)	Work wasted (%)	Duration (msec.)	Work wasted (%)	Duration (msec.)	Work wasted (%)
Locust able to start, 25° C.	49	25	58	51	69	88
Minimum for sustained flight, 31° C.	38	4	43	9	51	30
Intermediate for sustained flight, 35° C.	33	0	37	3	43	9
Maximum for sustained flight, 42° C.	23	0	26	0	31	0

Before flight starts the thoracic and ambient temperatures are equal, but during the first few minutes of flight the muscle temperature increases by 6–7° C. at ordinary lift and speed (Weis-Fogh & Muus, unpublished). At 22° C. the duration of a *twitch* equals the normal wingstroke period of an average female (59 msec.) so that more than 50 % of the mechanical work is being spent against the antagonists. After the thorax has heated up to 28° C., the duration is reduced to 43 msec. and the internal expenditure to only 6 % of the total work. Double firing would be wasteful, however, so that the locust would have considerably reduced reserves of mechanical energy. When the animal starts at 25° C., the first seconds will also be rather expensive but when the muscle temperature has risen above 30° C. both twitch activity and a moderate degree of double firing can ensure economical and sustained flight. Between 30 and 35° C. ambient temperature there are no problems with respect to work economy. Above 37° C. air temperature, flight deteriorates, one cause of which could be over-heating of the thorax since, between 43 and 45° C., the centrally disconnected motor nerve begins repetitive firing after a single stimulus (Fig. 8) and eventually dies. It is also worth noting that the results are in agreement with the observation that the flight performance is independent of the ambient temperature between 25 and 35° C., because neither twitch force nor tetanic force changed significantly between 25 and

42° C. muscle temperature. The main influence of temperature on locust flight may therefore be explained by the properties of the isolated wing muscle but the problem is not solved until we know why the activity of the central nervous system remains almost constant.

Comments on double firing. In vertebrates with many motor units in each muscle, double firing in the specific sense is probably of minor importance and in arthropods with a small number of units per muscle an exact control of double firing need not be essential provided that many muscles participate to produce a co-ordinated movement. However, the most important control muscles in a locust wing are the first basalar muscle with only one unit and the subalar muscle with two units (Wilson & Weis-Fogh, 1962). Fig. 6 shows that in such cases the amount of work may vary two
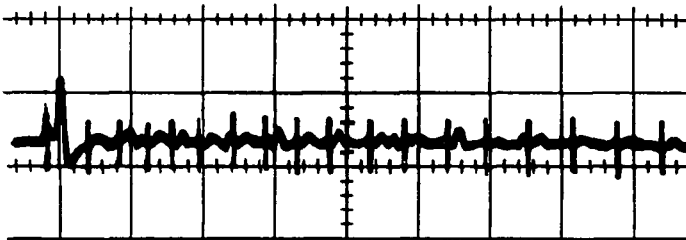

Fig. 8. Repetitive firing of a single motor unit in response to a single nerve stimulation, due to irreversable heat damage to the motor axon at 45° C. (saline).

to three times according to when the second firing occurs. Since it is known that the single motor neurons may act as independent oscillators (Wilson, 1961) it is worth pointing out that the timing of the second firing relative to the first could hardly be governed by a simple relaxation-oscillation system because, beyond the optimum for force and work, increasing excitation would then result in diminishing mechanical return. A more elaborate model is needed.

Remarks on speed. The speed of shortening of locust wing muscle is about equal to that of mammalian muscle and the most obvious specialization is therefore the early and quick relaxation which is responsible for the short twitch duration (Buchthal *et al.* 1957). According to the argument in this paper it is to be expected that the total twitch of *Hemaris* (bee hawk moth) and *Trochilium* (clearwing moth) wing muscle lasts only 5–6 msec., the wingstroke frequency of these moths being about 100 cyc./ sec. (Sotavalta, 1947). It would be interesting to study the sarcoplasmic reticulum in these muscles in view of its probable bearing upon the mechanism of relaxation (Porter, 1961). In the fastest muscle known so far (the swim bladder muscle of toad-fish; Skoglund, 1961), this structure reaches an exceptional degree of organization (Fawcett & Revel, 1961). As to the excitation-contraction coupling, an analysis of frog muscle similar to that in Fig. 3 C may be rewarding.

SUMMARY

Mechanical and electrical responses were studied in isolated nerve-muscle preparations at various temperatures, mainly in relation to current problems in locust flight.

1. The delay between the peak of the action potential and the onset of a twitch is

1 msec. and independent of temperature from 30 to 45° C. (the range encountered in sustained flight), indicating that physical rather than chemical processes are responsible for the main time-consuming links in the excitation-contraction coupling.

2. There is no mechanical staircase effect in a succession of twitches and no post-tetanic potentiation so that identical and closely spaced twitches, as well as doubly fired contractions, can follow each other in long trains.

3. Above 25° C., the tetanus:twitch ratio is 2 and the work is independent of temperature.

4. Two closely timed stimuli (double firing; 2–10 msec. interval) may result in 2 to 3 times more work than in a twitch. The increase is graded by the interval and the contraction is prolonged over that of the twitch only by this interval.

5. The twitch durations are smaller than previously reported so that the loss of energy due to interaction between antagonists is insignificant during normal flight, in both singly and doubly fired contractions.

6. The temperature-dependence of locust flight is in accordance with the pro-perties of the isolated nerve-muscle preparation.

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