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MECHANICAL POWER OUTPUT FROM STRIATED MUSCLE DURING CYCLIC CONTRACTION

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SUMMARY

1. The mechanical power output of a synchronous insect muscle was determined by measuring tension as the muscle was subjected to sinusoidal length change and stimuli which occurred at selected phases of the length cycle. The area of the loop formed by plotting muscle tension against length over a full cycle is the work done on that cycle; the work done times the cycle frequency is the mechanical power output. The muscle was a flight muscle of the tettigoniid *Neoconocephalus triops*. The measurements were made at the normal wing-stroke frequency for flight (25 Hz) and operating temperature (30 °C).

2. The power output with a single stimulus per cycle, optimal excursion amplitude, and optimal stimulus phase was $1{\cdot}52\ J\ kg^{-1}\ cycle^{-1}$ or $37\ W\ kg^{-1}$. The maximum power output occurs at a phase such that the onset of the twitch coincides with the onset of the shortening half of the length cycle. The optimum excursion amplitude was 5·5 % rest length; with greater excursion, work output declined because of decreasing muscle force associated with the more rapid shortening velocity.

3. Multiple stimulation per cycle increases the power output above that available with twitch contractions. In this muscle, the maximum mechanical power output at 25 Hz was $76\ W\ kg^{-1}$ which was achieved with three stimuli per cycle separated by 4-ms intervals and an excursion amplitude of 6·0 % rest length.

4. The maximum work output during the shortening of an isotonic twitch contraction was about the same as the work done over a full sinusoidal shortening-lengthening cycle with a single stimulus per cycle and optimum excursion amplitude and phase.

INTRODUCTION

The most important functional capacity of a muscle is its ability to shorten against a load and thus to do work. Despite its significance, work production by muscle is infrequently studied and poorly characterized, at least at cellular and tissue levels. There are many studies of tension produced by whole muscles or muscle fibres contracting at fixed length, a condition at which no external work is done, but there are few accounts describing work output by whole muscles or

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muscle fibres and fewer yet giving information on the sustainable rate of work output. In the literature dealing with sports medicine, biomechanics and ergonomics there are many accounts of mechanical work output by active humans. However, because of uncertainty about which muscles and which fibres within these muscles are involved in the activities considered and uncertainty about the extent to which voluntary exertion fully activates participating musculature, it is difficult to use information on work output by intact organisms to infer physiological properties of individual muscles and muscle fibres.

Perhaps the major reason that mechanical power output is infrequently studied in other than intact organisms is the lack of a realistic and realizable method for measuring mechanical work. Most direct measurements of muscle work have been with muscles shortening under constant load (isotonic contraction). But muscles *in vivo* rarely if ever operate at constant load, so isotonic measurements are not those of normal operating conditions. Further, most isotonic work studies have not considered the work which must be done on a muscle after it has shortened to restretch it to its original length, a factor whose magnitude may be appreciable at higher operating frequencies.

The following report considers the mechanical power output by a muscle subject to cyclic length change at a frequency similar to that of normal performance, and to stimulation at a selected phase in the length cycle. The approach is an extension of that used by Machin & Pringle (1960) to measure power output from asynchronous insect muscle during tetanic stimulation and, more recently, that used by several laboratories to measure work output of glycerinated or mechanically skinned muscle fibres under conditions of continuous activation (e.g. Jewell & Rüegg, 1966; Steiger & Rüegg, 1969; Kawai & Brandt, 1980). With the introduction of phasic stimulation, the approach becomes applicable to ordinary synchronous muscle and offers a realizable and realistic method for estimating the mechanical power output of muscle under nearly normal operating conditions.

Justification of the approach

Consider a muscle in a limb which is undergoing cyclic movement, for example a leg muscle in a walking or running animal. If the joint spanned by the muscle undergoes cyclic changes in angle, and if the tendons to which the muscle is attached are relatively non-compliant, then the muscle must undergo cyclic changes in length. If the limb movements are regular and of constant amplitude, then the length changes of the muscle must be regular and of constant amplitude, and this is true whether the muscle is stimulated and active or simply passively moved by the limb activity.

The power output of a muscle undergoing cyclic changes in length depends on when the muscle is active during the length cycle. If the muscle were a flexor, for example, its net work output per cycle would be positive (i.e. the muscle would do work on the limb) if the muscle developed tension during joint flexion and relaxed so as to offer little resistance to elongation during subsequent joint extension. Conversely, the work output over a full cycle would be negative (i.e. the limb would

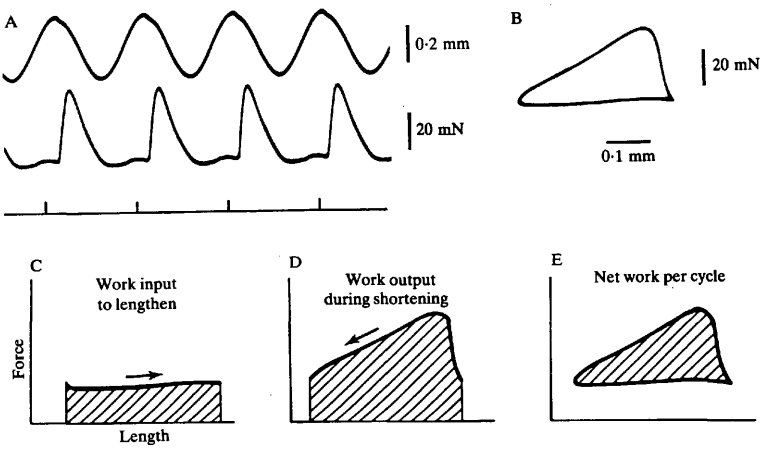

Fig. 1. The method for measuring work output. (A) A metathoracic first tergocoxal muscle is stimulated while it is subjected to sinusoidal length change. The upper trace monitors muscle length, the middle trace muscle tension, and the lowest trace time of stimulation. (B) Muscle force plotted against length. The area of the resulting loop is the work done per cycle (C)–(E).

do work on the muscle) if the muscle were relaxed during joint flexion and active, resisting elongation, during joint extension.

The work output per cycle can be determined from the area of the loop formed by plotting muscle force against length over a full cycle (Fig. 1). The area between the length axis and the limb of the loop formed by the lengthening phase, from minimum to maximum length, has units of work and is, in fact, the work required to stretch the muscle from its shortest to its longest length. Similarly, the area between the length axis and the shortening portion of the curve is the work done by the muscle during its shortening. The area of the loop itself is the difference between the work required to stretch out the muscle and the work produced during the subsequent shortening. This area then, is the work output per cycle (Fig. 1). It might be noted that the force-position display is a counterclockwise loop if the muscle does work upon its load and a clockwise loop if the load does work upon the muscle.

This approach was used to measure the mechanical work output of insect flight muscles subject to sinusoidal changes in length at the normal wing-stroke frequency. The measured parameter was work per cycle; the independent variables examined were amplitude of the cyclic length change, the phase between muscle activation and length change, and the number and pattern of stimuli used to activate the muscle on each cycle.

MATERIALS AND METHODS

The wing muscles were metathoracic, first tergocoxal muscles of the tettigoniid *Neoconocephalus triops*. The first tergocoxal muscle is a wing elevator. Adult, male animals were captured while they were singing in the early evening in fields and urban areas in Irvine, California. Animals were provided with water and fresh grass in the laboratory and were used within 3–4 days of capture.

The wing-stroke frequency of *N. triops* was determined earlier to be 23 Hz at an ambient temperature of 25 °C (Josephson, 1984). Because of heat production by the active wing muscles, the thoracic temperature during flight is certainly somewhat higher than the ambient temperature. The results described below were obtained at a contraction frequency of 25 Hz and a muscle temperature of 30 °C. These values were selected as being reasonably representative of normal flight.

An *in vivo* muscle was used in which the tracheal air supply to the muscle was intact and in which the muscle was bathed largely by normal haemolymph. After cutting the wings from an animal and the legs distal to the coxae, the metathoracic sternal plate was removed to expose the metathoracic ganglion. The ganglion was removed after severing all its nerves and connectives, thus denervating the metathoracic tergocoxal muscles. The metathoracic coxa on one side was freed from all attachments to the animal other than the apodeme of the tergocoxal muscle. This semi-isolated coxa was later used as the attachment point for the ergometer. The muscle was stimulated with 50 μm silver wires which were inserted into the dorsal attachment of the muscle through small holes in the exoskeleton and waxed in place. After the stimulating electrodes were in place, the animal was attached, ventral side up, to a Leucite platform with quick-setting epoxy cement. The cement solidly fixed the insect's tergum which is the dorsal attachment of the muscle. The platform was placed in a manipulator which allowed precise positioning of the preparation with respect to the ergometer. The muscle was stimulated with 0·5 ms shocks, usually at about twice the minimal intensity required to activate the three motor units of the muscle (Josephson, 1984). The preparation was moistened, as required, with saline (Usherwood 1968; pH adjusted to 7 with NaOH immediately before use). The temperature of the metathoracic segment was monitored with a thermocouple probe (0·3 mm o.d.) inserted into the contralateral tergocoxal muscle through the stump of the contralateral limb. The temperature was maintained at 30 °C by manually adjusting the intensity of a microscope lamp whose beam was centred on the ventral midline of the metathoracic segment.

The ergometer was a commercially-available instrument (Cambridge Technology 300H, Cambridge, MA. U.S.A.) which uses a galvanometer motor with capacitance position sensors for position control and force measurement. A locally-manufactured control circuit produced a sinusoidal voltage about zero which was used as a position control signal to the ergometer. The sinusoidal output was of variable amplitude, frequency and number of cycles. In order to avoid sudden position changes at the onset and termination of the sinusoidal movement, the sine waves always began and ended at the mid-point of the amplitude range, i.e. at zero voltage. The control circuit also generated a pulse on each cycle which was used to

trigger a stimulator. The phase between the trigger signal and the sine wave was continuously adjustable.

Experimental muscles were subjected to a burst of sinusoidal length change and phasic stimulation usually lasting five cycles. In the first experiments the muscle force and position were displayed on the Y and X axes of an oscilloscope and photographed. The photographed work loops were projected with an enlarger and traced, and the areas of the traced loops were determined with a digital planimeter. In later experiments, the force and position signals from the ergometer were digitized and analysed with a computer. The computer was an IBM PC; the analogue to digital converter was a Tecmar Lab Master (Tecmar, Cleveland, Ohio, U.S.A.). The sampling interval was 40 μs for a force-position pair and the digitized resolution was 12 bits. The computer determined the area of a pre-selected cycle, usually the fourth out of five. Because of facilitation of unusual degree or double firing by the muscle, on occasion the work loop for the cycle of interest did not close and the values for force or position at the end of the cycle were substantially different from those at the onset of the cycle. Loops were discarded if the differences between the initial and final force measurements for the cycle exceeded 5 % of the total force range or if the difference between the initial and final position values exceeded 5 % of the position range. Using the computer for analysis resulted in a substantial reduction of measurement error, and the values reported in this paper were all obtained using the computer system. (Additional information on the control circuit and the hardware and software used in the computer analysis is available on request.)

Because the experimental muscle was nearly totally enclosed by the animal, it was not possible to measure directly the muscle length during an experiment. Before an experiment the muscle length was adjusted by positioning the manipulator holding the preparation until the muscle was under slight tension and the exposed ventral portion of the muscle seemed to be appropriately orientated with respect to adjacent cuticular structures. At the end of an experiment the animal was injected with 70 % ethanol to fix histologically the muscle in place before it was detached from the ergometer. The preparation was then stored for several days in 70 % alcohol before it was dissected. The experimental muscle and its contralateral control, fixed *in situ* attached to its coxa, were dissected free and their lengths measured with an ocular micrometer. Preparations in which the length of the experimental muscle differed from that of the control by more than 10 % were not included in the analysis. After the experimental muscle had been cleaned of adhering extraneous tissue, it was rehydrated in insect saline for several hours, blotted dry, and weighed with a torsion balance to the nearest 0·01 mg. Wing muscles from the tettigoniid *N. robustus* lose about 12 % of their original wet weight if they are fixed and stored in 70 % alcohol (Ready, 1983). It was assumed that there is a similar weight loss in muscles of *N. triops*. Correction was made for the assumed weight loss in calculating the original muscle mass. Muscle cross sectional area was determined as the ratio of muscle weight to length. A total of 27 successful preparations was used. In these, the average muscle length was 6·10 mm (S.D. = 0·31 mm), the average mass 5·53 mg (S.D. = 0·08 mg) and the average cross-sectional area 0·0091 mm^2 (S.D. = 0·0012 mm^2).

The phase between muscle activation and length is expressed below in terms of the delay between the time of maximum muscle length and the projected time of peak isometric twitch force. Specifically, phase (%) = 100×(time of projected maximum isometric twitch tension minus time of maximum length)/cycle length. Force was measured isometrically at the beginning of experimentation with each preparation and the delay between a suprathreshold stimulus and the resulting twitch peak was determined. This delay, generally 10–12 ms, was added to the time of stimulation in the work measurements to obtain the projected time of peak isometric twitch force used in phase calculations. A phase of 0 indicates that the maximum length coincides with the expected time of maximum isometric twitch tension. Negative phase (0 to −50 %) indicates that the time of projected maximum force came before the maximum length, i.e. during the lengthening portion of the cycle; while a positive phase (0 to +50 %) indicates that the projected peak tension came after the maximum length, during the shortening portion of the cycle. The same convention is used to express phase for bursts of stimuli per cycle. With multiple stimuli, a phase of 0 indicates that the maximum muscle length coincides with the time of projected maximum twitch force evoked by the first stimulus of the burst.

Action potentials from wing muscles of flying animals were recorded with silver wires, 50 μm in diameter and insulated to the tip, which were inserted into the tergocoxal muscles through small holes in the exoskeleton. The electrodes were fixed in place with cyanoacrylate cement. After placement of the electrodes, the animal was fixed to a holder by its notal shield and flight was initiated by blowing a stream of air caudally across the animal's head. Muscle action potentials were amplified with capacitor-coupled amplifiers, displayed on an oscilloscope, and photographed.

RESULTS

Work output with one stimulus per cycle

Intertrial interval

In the metathoracic tergocoxal muscle of *N. triops* the work output on a given cycle of a trial at optimum excursion amplitude and phase is greater the greater the intertrial interval. This is true for intervals up to at least several minutes. If a well-rested muscle is subjected to a series of trials at constant intertrial interval, each trial consisting of 4–5 cycles of length change and stimulation, the work output per cycle declines on successive trials until a steady state is reached. The value of the steady state is smaller the shorter the intertrial interval down to intervals of several seconds which were the shortest tested. Except where otherwise indicated, the results below were all from intertrial intervals of 30 s at steady state. Thirty seconds as an intertrial interval was selected as a compromise between intertrial intervals of several minutes which might give more work and intertrial intervals of several seconds which would allow examination of many combinations of parameters before being limited by deterioration of the preparation. In the 11 preparations in which it was specifically examined, the steady-state work per cycle at optimum excursion

amplitude, optimum phase and an intertrial interval of 30 s averaged 90·4 % (S.D. = 5·8 %) of the work per cycle under the same conditions but on the first trial after a 5-min rest period.

There are both facilitative and depressive components to the influences of interstimulus interval and intertrial interval on work output. The work per cycle generally increases over the first 2–3 cycles of a trial, after which the work output becomes essentially constant and the successive work loops are superimposable. This initial increase in the work per cycle during a trial is due to short-term facilitation. The facilitation must decay rapidly since the responses on the next trial are again initially smaller than the steady-state value, even if the intertrial interval is only a few seconds long. In addition to the short-term facilitation, there can be long-term augmentation analogous to post-tetanic potentiation. Following presentation of trials with 3–5 stimuli per cycle, the work output on trials with a single stimulus per cycle is increased over steady-state levels for several minutes. I have not yet fully characterized the short- and long-term augmentation of work output or the long-term depression seen as declining work output during successive trials until the achievement of steady-state.

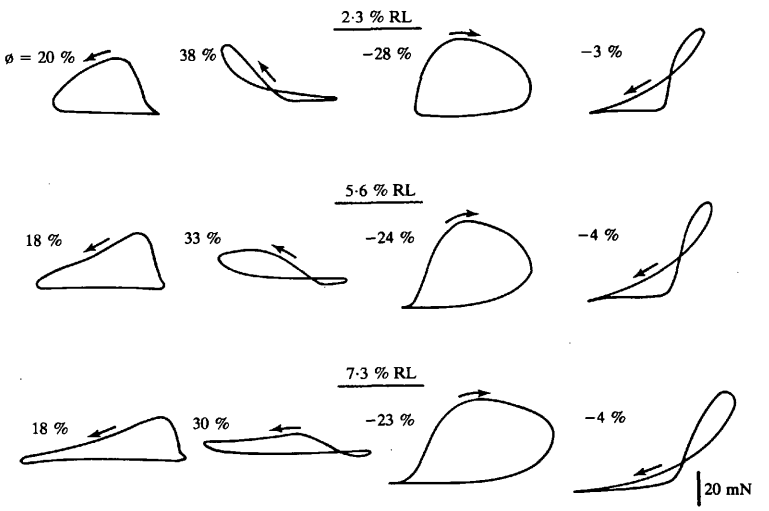

Fig. 2. Work loops at different excursion amplitudes (measured peak-to-peak as percentage of rest length, RL) and stimulus phase. The counterclockwise loops in the left column are for the stimulus phase giving maximum work output by the muscle. The clockwise loops in the third column are for the stimulus phase at which the work done on the muscle was maximum. The second and fourth columns are intermediate stimulus phases during which work was done by the muscle during part of the cycle and on the muscle for the rest of the cycle. The force scale (ordinate) is the same for all loops. The length scale (abcissa) was adjusted with each change in excursion amplitude so as to obtain similar displays.

Phase, excursion amplitude

If the phase between a stimulus and muscle position is such that the maximum muscle force occurs near the middle of the shortening portion of the cycle, the force-position loop is traversed in a counterclockwise direction, showing that the work output is positive with the muscle doing work on the apparatus. If the peak force comes near the middle of the lengthening portion of the cycle, the force-position loop is traversed clockwise and the work is negative, i.e. the apparatus does work on the muscle. At intermediate phases the work loop can be complex with work being positive during some parts of the cycle and negative in other portions (Fig. 2).

The work output strongly depends on the amplitude of the sinusoidal length change. In the part of the stimulus phase range characterized by negative work, the absolute magnitude of the negative work increases continuously with increasing amplitude of the length oscillation (Fig. 3). The effects of excursion amplitude in the phase range characterized by positive work are complex. As the amplitude of the length oscillation is increased, the range of stimulus phase which gives positive work initially increases but then becomes increasingly narrow (Fig. 3). In addition, the work output at optimum phase initially increases with increasing excursion amplitude but then reaches a maximum and subsequently declines (Figs 3, 4). The initial increase in work output reflects increasing area of the work loop because of broadening along the position axis. The later decline in work output with increasing amplitude of the length oscillation is a reduction in the area of the work loop because of narrowing along the force axis. The muscle cannot shorten rapidly enough to maintain high tension during the rapid shortening associated with large amplitude position oscillations and, as a result of the reduced force, the work per cycle declines.

In a set of preparations in which the conditions giving maximum work output for single stimuli were deliberately sought, the peak work per cycle averaged $1{\cdot}52\,J\,kg^{-1}$ (S.E.M. = 0·08, $N = 22$). At the cycle frequency of 25 Hz this equals $38{\cdot}0\,W\,kg^{-1}$. These values are for one trial every 30 s at steady state; the work per cycle on single trials after a long rest is somewhat greater. The maximum work at steady state was achieved at a peak-to-peak amplitude excursion of 5·65 % rest length (S.E.M. = 0·15 %) and a phase of 19·4 % (S.E.M. = 0·5 %). The phase of 19·4 % is equivalent to a delay between the start of shortening and the projected time of peak isometric force of 7·8 ms. Interestingly, the twitch rise time for this muscle at 30 °C was earlier determined to be also 7·8 ms (Josephson, 1984). Thus for maximum work at optimum amplitude of length oscillation the onset of a twitch contraction should occur at the onset of the shortening phase of the cycle. The phase giving maximum work varies somewhat with the amplitude of the length oscillation, becoming smaller the larger the oscillation (Fig. 3).

Length oscillation without stimuli; stimuli without oscillation

Imposing a sinusoidal change in length upon an unstimulated muscle requires work being done on the muscle. The position-force loops generated by an un-

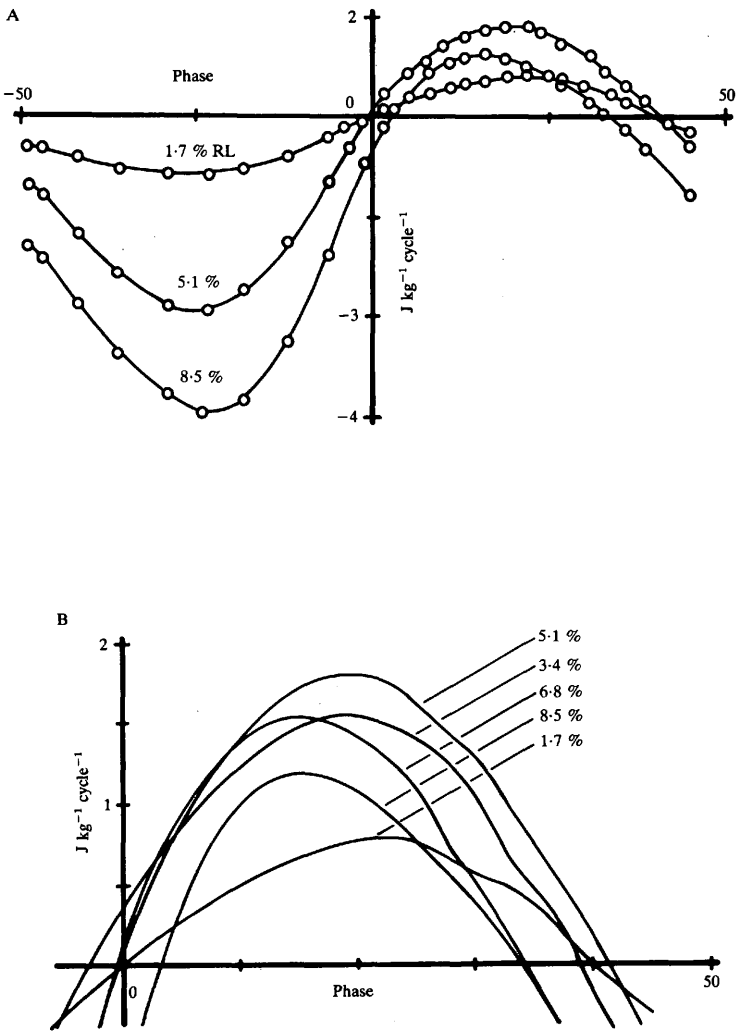

Fig. 3. Work output as a function of excursion amplitude (% rest length, RL) and stimulus phase. The data are all from a single preparation. The individual datum points are shown in A but, for clarity, have been omitted in B which is the work output in the positive range.

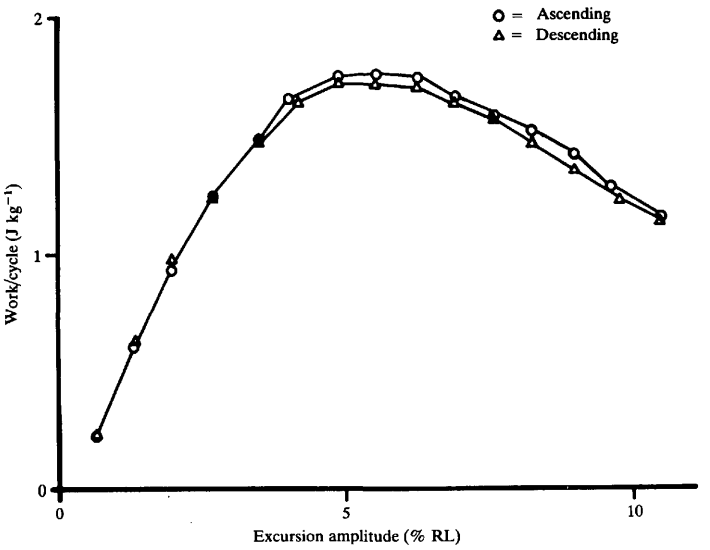

Fig. 4. Work output at optimum phase as a function of excursion amplitude. The points shown are from a series of measurements at increasing amplitude excursion immediately followed by a similar series at decreasing excursion. The stimulus phase was 18% throughout. RL, resting length.

stimulated muscle are clockwise, confirming that the muscle is absorbing rather than producing work. The magnitude of the work absorbed by an unstimulated muscle is a non-linear function of the oscillation amplitude. At a peak-to-peak amplitude of 5–6% rest length (the excursion amplitude giving maximum work in a stimulated muscle) the work absorbed per cycle is 0·1–0·25 J kg^{-1}, which is about one-tenth of the work produced by a muscle stimulated once each cycle at optimum conditions of length oscillation and phase. Part of the work absorbed by an unstimulated muscle is presumably dissipated by the inactive contractile components, part by viscous resistance of the apodeme, cuticle and other damped series compliance elements. The non-contractile sinks for work are probably present as work-absorbing components in active as well as passive muscle. Thus, some fraction of the work output of the contractile component is probably lost in viscous series compliance elements. The fraction of the work output which is lost is probably small, judging by the relatively small amount of work absorbed by a passive muscle subject to oscillatory length change.

Using this ergometer, stimulation of a muscle in the absence of a sinusoidal input position signal, and therefore with no supposed movement of the recording lever, resulted in small, clockwise work loops. This work was presumably a consequence of tracking error by the servosystem of the ergometer. Were the ergometer truly

isometric, the work per cycle would be zero; in actuality there was some work done. The work was always negative and greater the greater the force generated by the muscle. Typically the negative work during 'isometric' twitch contractions of a muscle was 10–20 % as large as the positive work from the muscle at optimum phase and excursion amplitude. This negative work, which I assume is due to inadequacies of the ergometer, is likely to be present during the usual work measurements. Therefore the positive work during sinusoidal length change may be slightly underestimated. The measurement error is probably not very great, especially given that during conditions giving maximum positive work the muscle is shortening rather rapidly at the time of peak muscle tension and the force upon the ergometer is 10–40 % less than that during isometric twitch contractions.

Work output during isotonic twitches

A more usual method for determining work output during twitch contractions is to measure the distance of shortening during isotonic twitches. The shortening distance during isotonic twitch contractions is inversely related to muscle load (Figs 5, 6). The work output during the shortening phase, which is the product of force and shortening distance, is a bell-shaped function of load; being zero both at zero load and at the maximum isometric twitch tension, and having a maximum at some intermediate load (Fig. 6).

In six preparations, work output was first determined from isotonic twitches as in Fig. 6 and then using sinusoidal length change at 25 Hz. The interstimulus interval was not carefully controlled in the isotonic twitch sequences but was of the order of 4–10 s. Each trial in the oscillatory work determinations included five stimuli and

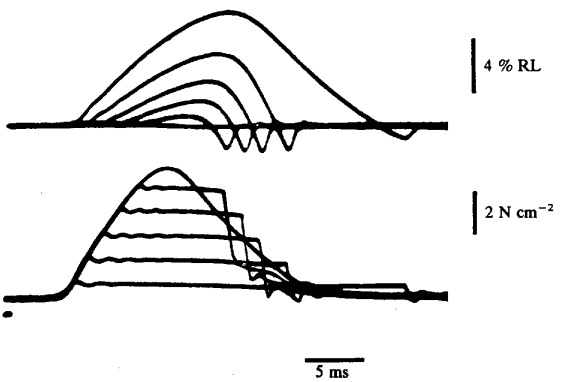

Fig. 5. Force (lower set of traces) and shortening (upper traces) during after-loaded, isotonic twitches. The small oscillations in the force record are a consequence of under-damping of the ergometer. RL, resting length.

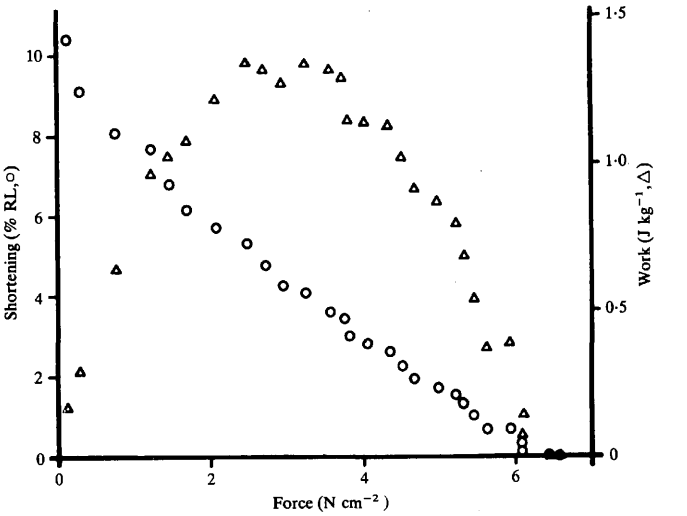

Fig. 6. Muscle shortening (○) and work (△) in after-loaded isotonic contractions. The data are all from the experiment shown in part in Fig. 5. The work is the product of force and shortening distance and is thus the work done by the muscle during the shortening phase without allowance for the work done on the muscle by the load during relaxation. RL, resting length.

the intertrial interval was 30 s, so the overall rate of stimulation was similar in the two methods of work determination. The maximum work output during isotonic twitches averaged 1·30 J kg^{-1} (S.E.M. = 0·09) while the work output per cycle during oscillatory length change at optimum excursion amplitude and phase averaged 1·43 J kg^{-1} (S.E.M. = 0·11). In these preparations the optimum load for work during isotonic twitches allowed muscle shortening of 4·4% rest length (S.E.M. = 0·3%) while the optimum excursion amplitude for work output during sinusoidal oscillation was 5·0% (S.E.M. = 0·2%). The differences in work output and optimum amplitude between the two approaches were not statistically significant ($P > 0·05$, two-tailed t-test for paired samples).

Multiple activation per cycle

Muscle activity patterns during flight

Muscle action potentials recorded from metathoracic, first tergocoxal muscles during tethered flight sometimes occur as single peaks per wing cycle (Fig. 7), indicating that the motor units contributing to the recorded activity are active once, and roughly synchronously, during the cycle. The sampled motor units in these cycles are operating with single twitch contractions. More often, however, the recorded muscle action potentials have two, three or four peaks per wing cycle

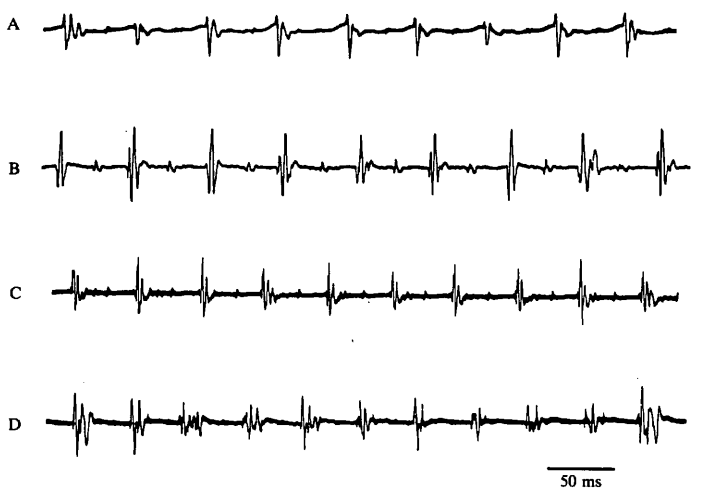

Fig. 7. Muscle action potentials recorded from the metathoracic first tergocoxal muscle during tethered flight. (A)–(D) are segments of flight records from four different animals.

(Fig. 7). Some of the multiple peaks may be due to asynchrony in the activation of the three motor units making up the muscle (Josephson, 1984), but the activity pattern often strongly suggests that there is multiple firing of at least some of the motor units. The measurements below were done to determine the work output attainable with multiple activation per wing cycle.

Work output with multiple stimuli per cycle

Work output during sinusoidal length change at 25 Hz was determined using two, three, four and five stimuli per cycle and interstimulus intervals graded in 2-ms steps. All combinations of stimulus number and interval were tested in which the duration of the stimulation period was 10 ms or less with two stimuli per cycle and less than 20 ms with three, four and five stimuli per cycle. Each trial consisted of four to five cycles and the intertrial interval was maintained at 30 s.

The work at optimum phase and excursion amplitude was systematically determined for each stimulus condition in the following manner. An optimum excursion amplitude was estimated, and the phase giving maximum work was determined in an ascending and then a descending series of trials in which the phase was varied in steps of about 4 %. The starting point for each series was chosen so that the series passed through the optimum phase. The sequence was repeated if the maximum work on the ascending and descending series did not occur on the same or adjacent phase steps. The phase was then set at that giving maximum work and the optimum

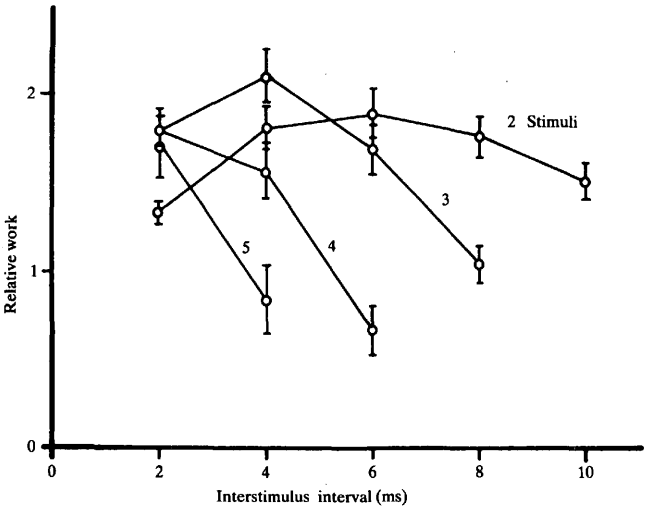

Fig. 8. The effect of stimulus number (number beside each trace) and frequency on work output. With each stimulus configuration, the maximum work per cycle, the optimum excursion amplitude and the optimum phase were systematically determined (see text for details). The work output is expressed as the ratio of the maximum work with the given stimulus conditions and the maximum work with a single stimulus per cycle as determined either immediately before or immediately after the determination with repetitive stimuli. Thus a relative work of 2 indicates that the maximum work with multiple stimuli was twice that with single stimuli. The data are shown as mean ± standard error ($N = 6$).

excursion amplitude was determined in an ascending and descending series in which the amplitude was varied in steps of about 0·7% rest length. The determination of optimum excursion amplitude was repeated if the maximum work did not occur on the same or on adjacent amplitude steps in the ascending and descending series. If the newly-determined optimum amplitude differed by more than one amplitude step from that used earlier in determining the optimum phase, the new optimum amplitude was used and the optimum phase was redetermined.

A full set of determinations in these measurements required several hours, during which time there was sometimes significant change in the condition of the preparation. In order continually to assay the state of the muscle, each determination of work output with multiple stimuli was either immediately preceded by or immediately followed by a determination of work output with single stimuli per cycle at optimum phase and excursion amplitude determined as with the multiple stimuli. Preparations were discarded in which the work output to single stimuli declined by more than 20% in the series. A total of six successful preparations was obtained. In four of the preparations the series began with two stimuli at an interstimulus interval of 2 ms, progressed systematically through paired stimuli at

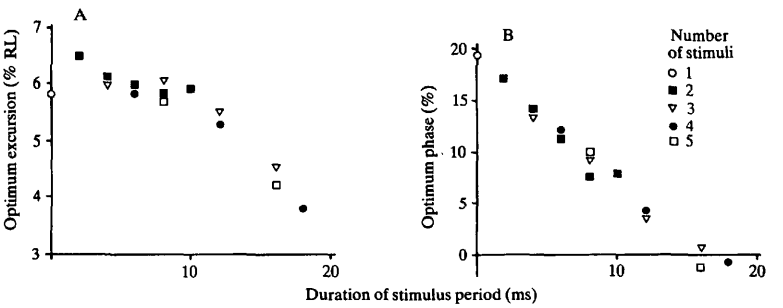

Fig. 9. The excursion amplitude and the stimulus phase which give maximum work per cycle as functions of the stimulus number and frequency. The abcissa is the total duration of the stimulation period per cycle, given as the product of the interstimulus period and the number of interstimulus intervals. RL, resting length.

increasing interstimulus intervals to trials with three, four and five stimuli, and ended with five stimuli at 4-ms interstimulus intervals. In the other two preparations, the series was in the opposite order, beginning with five stimuli at 4-ms intervals and ending with a pair of stimuli at a 2-ms interval.

The maximum work output with multiple stimuli per cycle is roughly twice that available with single stimuli (Fig. 8). The stimulus conditions giving maximum work varied from preparation to preparation. In two of the six preparations used and in the average of all preparations, the work was maximum with three shocks at 4-ms intervals. In two preparations, the work was greatest with two shocks at 6-ms intervals. In the remaining two preparations, the work was maximum with four and five shocks at interstimulus intervals of 2 ms. The work output per cycle at optimal conditions in the six preparations averaged $3{\cdot}03\,J\,kg^{-1}$ (S.E.M. = 0·48) which is a power output of $75{\cdot}8\,W\,kg^{-1}$.

The sinusoidal excursion amplitude at which work is maximal is not strongly dependent on the number or the pattern of activating stimuli, so long as the total period of stimulation is 10 ms or less (Fig. 9A). The optimum excursion amplitude is essentially the same for single stimuli as for short bursts of stimuli which produce up to twice as much work per cycle. Increasing the total period of stimulation beyond 10 ms, either by increasing the number of stimuli or by increasing the interstimulus interval, results in both a decrease in mechanical power output (Fig. 8) and a decrease in the optimum amplitude (Fig. 9A).

The stimulus phase at which work is maximum decreases linearly with increasing duration of the stimulation period (Fig. 9B). The slope of the relationship between phase and stimulation period is $-1{\cdot}17$ % per ms. Since the cycle duration is 40 ms, this slope is a decrease in the time of the onset of stimulation of 0·47 ms per ms increase in the duration of the stimulation period. This phase shift is almost exactly that which would be required (0·5 ms per ms) to keep the mid-point of the stimulation period at a fixed point in the cycle. Thus, the optimum phase for any

duration of stimulation is apparently one which results in the stimulation period being placed symmetrically about the optimum phase for single stimuli.

DISCUSSION

The maximum power output of muscle

The maximum mechanical power output of metathoracic wing muscles of *N. triops* during oscillatory contraction at 25 Hz and 30 °C, with optimum amplitude and stimulus parameters, is 76 W kg^{-1} muscle. As compared to most striated muscle, the wing muscles of insects have unusually abundant mitochondria and sarcoplasmic reticulum (e.g. Elder, 1975). Therefore the fraction of the wing muscle mass which is myofibril, the component which produces mechanical power, is comparatively low. The muscles of *N. triops* have not been examined with an electron microscope. In *N. robustus*, a close relative of *N. triops* with nearly identical wing muscle contraction kinetics (Josephson, 1984), myofibrils make up about 57% of the volume of metathoracic wing muscle fibres (Ready, 1983). Assuming that the ultrastructural organization of muscles is similar in *N. triops* and *N. robustus*, and that the volume of components other than muscle fibres in a muscle is negligible, the maximum mechanical power output from *N. triops* muscles is 133 W kg^{-1} myofibril.

The measured power output from wing muscles of *N. triops* is within the range of that measured from, or estimated for, other active muscles; but, because of the diversity of approaches which have been used and of the assumptions which have had to be made, the range of values for measured or estimated power output is so large that not to fall within it would in itself be remarkable.

The maximum continuous power output from athletic humans ranges from about 1500 W for very short bursts of activity to 370 W for activities sustainable for tens of minutes (Wilkie, 1960). Assuming a 60 kg body weight, this is a power output of 6–25 W kg^{-1} body weight. For a number of walking and running animals, the sum of positive increments of potential and kinetic energy gives a total of up to 20 W kg^{-1} body weight which is supplied by muscle work and elastic recoil from previous movement (Heglund, Fedak, Taylor & Cavagna, 1982). Hovering birds, bats and insects are estimated to expend 13–50 W kg^{-1} body weight (Weis-Fogh, 1973; Casey, 1981). In each of these examples, the work output per kg active muscle must be several times larger than the values for work per kg whole body weight.

Human muscles can deliver 100–400 W kg^{-1} muscle for periods of a few seconds when contracting against an inertial load (Hill, 1922; Lupton, 1923). The peak power output of tetanically stimulated muscle contracting against an isotonic load, as calculated from parameters of force-velocity relations, are: 1·8 W kg^{-1} for tortoise leg muscle at 0 °C (Woledge, 1968); 17 W kg^{-1} for frog sartorius muscle at 0 °C (Hill, 1938); 316 W kg^{-1} for frog semitendinosus muscle fibres at 20 °C (Cecchi, Colomo & Lombardi, 1978); 220 W kg^{-1} for the iliofibularis muscle of the lizard *Dipsosaurus* at 30 °C (R. Marsh & A. Bennett, in preparation); 266 W kg^{-1} for the metathoracic wing muscle of *N. triops* at 30 °C (Josephson, 1984) and 140, 225 and 469 W kg^{-1} for mouse soleus, inferior rectus and extensor digitorum longus

muscles, all at 35 °C (Luff, 1981). Assuming that the muscles in all these examples normally work in antagonistic sets and that each set is active over one-half the operating cycle, the power output for a muscle over a full cycle would be no more than half (and probably much less) than the values given.

Tetanically-stimulated beetle asynchronous muscle oscillating against an inertial load produced a sustained power output of up to 30 W kg^{-1} muscle, bumblebee muscle up to 60 W kg^{-1}, at estimated temperatures of 30–35 °C (Machin & Pringle, 1959; Pringle & Tregear, 1969). The greatest power output from glycerinated muscle fibres of the bug *Lethocerus*, sinusoidally-oscillated in an activating solution at 20 °C, was 7·6 W kg^{-1} (Pringle & Tregear, 1969). The necessity of relying on diffusion from the medium to deliver substrate probably limits power output from glycerinated fibres to values considerably less than is achieved *in vivo*.

Muscle power and flight

Flight is among the most energy-expensive activities of animals and the specific mechanical power output of flight muscle can be expected to be near the maximum of which striated muscle is capable. Weis-Fogh (1973, 1977) has estimated the sustainable mechanical power of aerobic flight muscle to be 60–360 W kg^{-1} muscle based on an aerodynamic model of flight performance, or on measured values of activity metabolism during flight and an assumed metabolic to mechanical efficiency by muscle of 0·2. Ellington (1984*b*), from aerodynamic measurements, estimates the mechanical power output of insect wing muscle during hovering flight to be 70–190 W kg^{-1}. Pennycuick & Rezende (1984), using a rather speculative model, hypothesize that aerobic flight muscles should be able to deliver a mechanical power output of more than 800 W kg^{-1} at very high operating frequencies. The maximum mechanical power output from flight muscle of *N. triops* during sinusoidal oscillation was 76 W kg^{-1}. This is within Weis-Fogh's and Ellington's ranges of estimated power output for wing muscles but clearly at the very lowest end of the ranges. *N. triops* does fly and does fly quite well, albeit usually for only short distances. It is thus a problem as to why the work obtainable from flight muscles of *N. triops* is at the low end of that predicted to be obtainable from flight muscle. It is possible, of course, that the technique used to measure power from *N. triops* muscles underestimates the *in vivo* performance. One potential source of difference between the *in vivo* performance and that actually measured is the trajectory of the cyclic change in muscle length, which was assumed in the work measurements to be sinusoidal. Wing movements of insects in flight are approximately sinusoidal (Weis-Fogh, 1956; Wilson & Weis-Fogh, 1962; Ellington, 1984*a*) but the basal articulation of the wings may be mechanically quite complex (Boettiger, 1957), so the length trajectory of the wing muscle could conceivably be quite non-sinusoidal during flight. If so, and if an appropriate non-sinusoidal length trajectory gives greater power output than a sinusoidal one, the use of an assumed sinusoidal trajectory would underestimate power output. On the other hand, it is also possible that Weis-Fogh's predicted mechanical power outputs of wing muscles during flight are overestimates. The aerodynamics of flapping flight are quite complex and a number of assumptions must be made in order to calculate mechanical power requirements, inaccuracies in any of which might affect the validity of the results.

And if the metabolic to mechanical efficiency *in vivo* is less than 0·2, as has recently been suggested by Ellington (1984*b*), using this value would overestimate the mechanical power output. The seeming discrepancy between predicted and measured power output by flight muscles should be a warning of possible inadequacies in either the theoretical or the empirical approaches.

Isotonic work and sinusoidal work

In the metathoracic wing muscles of *N. triops*, the work output during isotonic twitch shortening under optimum load ($1{\cdot}3\ J\ kg^{-1}$) is quite similar to the maximum work per cycle during sinusoidal oscillation at the flight frequency with one stimulus per cycle. This similarity in work output is a bit misleading, however. The work measured during isotonic twitches, unlike that during sinusoidal oscillation, is that produced during the shortening phase only and no allowance is made for any work which must be put into the muscle to re-lengthen it. The isotonic twitch work from flight muscles of *N. triops* is less than that reported for flight muscles of the locust during isotonic twitch contraction (up to $4{\cdot}5\ J\ kg^{-1}$ at 32 °C, Buchthal, Weis-Fogh & Rosenfalck, 1957), and less than that obtained from locust flight muscle during twitch contractions against a stiff spring ($5{\cdot}9\ J\ kg^{-1}$) at 36 °C (Neville, 1963; calculated assuming a muscle mass of 11 mg as in Buchthal *et al.* 1957).

Gradation of power output

Individual motor units in the metathoracic tergocoxal muscle of a flying katydid fire once to several times per wing cycle. Presumably in intact animals as in isolated muscles (Fig. 8), multiple firing is associated with increased power output. This is certainly so in the locust *Schistocerca gregaria*. The motor unit firing patterns during flight in *S. gregaria* are quite similar to those in the katydid *N. triops*. In *S. gregaria*, increasing lift during flight is associated with an increase in the number of active units in each muscle and an increase in the amount of repetitive firing to each motor unit (Wilson & Weis-Fogh, 1962). In both the locust and the katydid the extra work with repetitive activation above that obtainable with single stimuli is moderately large. Double firing by flight muscles of *S. gregaria* contracting against a spring increases the muscle work output by 2–3 times that obtainable with a single twitch (Neville & Weis-Fogh, 1963). In the flight muscles of *N. triops*, double firing can nearly double the work output as compared to that obtainable from the muscle with single stimuli and with three appropriately-timed stimuli the work output per cycle is more than doubled (Fig. 8).

The first tergocoxal muscle of *N. triops* is one of five pairs of major wing elevator muscles in the metathorax and there are three pairs of major depressor muscles. Each of these major flight muscles is composed of 2–5 motor units. The presence of multiple synergistic muscles for each wing, multiple motor units per muscle, and variable power output per motor unit based on activation pattern, should allow rather fine gradation of power output to meet the requirements of flight.

The optimum excursion amplitude for power output is essentially the same for single stimuli and for bursts of 2–5 stimuli whose duration is up to 10 ms and for which the work output per cycle is up to two times that for single stimuli. If performance *in vivo* is similar to that under the experimental conditions, one would

predict that increased power output by the muscles during flight, which would appear as increased lift or thrust, would be accomplished by changes in the angle of attack of the wings and not by changes in wing-stroke amplitude. In this respect, it is of interest that in locust flight, wing-stroke amplitude is nearly constant and independent of lift (Weis-Fogh, 1956; Wilson & Weis-Fogh, 1962).

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