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THE EFFICIENCY OF A FLIGHT MUSCLE FROM THE LOCUST *SCHISTOCERCA AMERICANA*

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SUMMARY

1. The efficiency of the metathoracic tergosternal muscle of the locust *Schistocerca americana* was examined by simultaneously measuring work output from the muscle and oxygen consumption by the muscle. The work output was determined using the work-loop technique in which the muscle is subjected to periodic strain and to phasic stimulation in the strain cycle. The area of the loop formed by plotting muscle force against muscle length over a cycle is the net work output for that cycle.

2. The tergosternal muscle is a synchronous, parallel-fibred muscle containing two motor units with similar contraction kinetics. The average twitch rise time (30 °C) was 15 ms, the twitch duration (to 50% relaxation) was 26 ms, and the peak twitch tension with both units active was 73 kN m^{-2}. The maximum mechanical power output during sinusoidal shortening at 20 Hz with both motor units active and stimulated once per cycle averaged 37 W kg^{-1}.

3. The overall efficiency of the tergosternal muscle averaged 6·4% (range 4–10%) where efficiency is defined as the ratio of the net work done (20 Hz sinusoidal strain, 1 stimulus per cycle, optimum strain amplitude and stimulus phase) to the caloric equivalent of the oxygen consumed. The efficiency was independent of the duration of the test period (examined range = 10–30 s) and the same when both motor units were active as when only one was stimulated.

4. Stimulating the muscle with two stimuli per cycle (interstimulus interval = 6 ms) increased the work per cycle by about 13% above that with single stimuli per cycle, but the muscle fatigued more rapidly and after 15–25 s the power output was less with two stimuli per cycle than with one. The efficiency with two stimuli per cycle was slightly less than that with one shock per cycle.

5. The oxygen consumption during normal work cycles at 20 Hz with optimum stimulus phase and strain was greater by about 15% than the oxygen consumption during isometric contractions at the same frequency.

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INTRODUCTION

Several recent reports suggest that the efficiency of insect flight muscles at converting metabolic energy into mechanical work is substantially lower than efficiency values commonly reported for vertebrate muscles (Ellington, 1984, 1985; Casey & Ellington, 1989; Stevenson & Josephson, 1990). Most estimates of the efficiency of insect flight muscles are based on measured oxygen consumption during flight and the calculated mechanical power requirements for flight. There are significant uncertainties in the calculations of the required mechanical power, in particular in the extent to which the kinetic energy of a moving wing can be stored elastically at the end of the wing stroke and used to accelerate the wings in the other direction at the onset of the next stroke. Because of these uncertainties, the range in estimates of efficiency for insect flight muscles is quite large.

The following is a direct determination of muscle efficiency in an insect muscle. The mechanical work output was measured from a single flight muscle operating under conditions which mimic those of *in vivo* performance, and the energy input was estimated from the increased oxygen consumption associated with the activity of that muscle.

The measurements take advantage of three features of insect flight muscles which make them particularly suitable for studies of work output and efficiency. First, most insect flight muscles appear to be of homogeneous fibre composition. The wing muscles of insects are made up of only 1–6 motor units (Kammer, 1985). Where there is more than one motor unit, the separate motor units are usually similar in contraction kinetics and in fibre ultrastructure (e.g. Josephson, 1973; Mizisin & Ready, 1986; Stokes, 1987). Because of fibre homogeneity, work output by the population of fibres making up a whole muscle is not degraded by simultaneous activity in fibres which differ in force–velocity characteristics or in the time course of activation. Second, the oxygen supply to insect muscles is through hollow tubes (trachea) which open to the exterior and whose terminations ramify throughout the tissues, including the muscles. In insect preparations it is possible to keep the tracheal system intact and to maintain the oxygen supply without having to keep intact also a circulatory system and lungs or gills. Finally, oxygen consumption is a good reflection of total metabolic rate in insect flight muscles, because these are aerobic tissues with little capacity for anaerobic metabolism (Beenakkers, Van der Horst & Van Marrewijk, 1985).

Oxygen consumption is rather far removed from the action of muscle cross-bridges and chemico–mechanical energy transduction by muscle. Efficiency based on oxygen consumption includes the efficiency of producing ATP from metabolic substrates and the efficiency of myofilaments in using ATP to do work; and it includes the costs of muscle activation and inactivation as well as the costs of cross-bridge cycling. However, it is the overall efficiency, that for which oxygen consumption is a good measure of energy input, which determines how much work can be done for a given amount of stored fuel; and it is this measure of efficiency that is most useful in evaluating limits to performance and the costs of activity during normal behaviour by animals.

METHODS

The preparation

The muscle used was the metathoracic tergosternal muscle (muscle 113; see Snodgrass, 1929), an indirect wing elevator, from the locust *Schistocerca americana*. This muscle, like the other skeletal muscles of a locust, is a synchronous muscle. Each muscle contraction is initiated by a muscle action potential or a burst of action potentials. The muscle is composed of parallel fibres that run from the dorsal tergum to the ventral sternum. The muscle is about 8·6 mm long and weighs about 30 mg. It was selected because of its relatively large size, and because it has a rather broad ventral insertion whose position is clearly marked by darker pigmentation in the overlying cuticle. The clear delineation of the muscle insertion made it possible to insert stimulating electrodes accurately through the ventral cuticle into the muscle, reducing the risk that the electrodes would lie close enough to adjacent muscles to inadvertently stimulate them.

To prepare a muscle for mechanical recording, the animal was first neurologically decapitated by cutting the thin cuticle of the neck to expose the ventral nerve cords, and then cutting the cords. The gut was ligated and transected in the neck and removed through a slit in the abdomen. The legs and wings were removed. Cuticular patches overlying the thoracic ganglia were removed, the nerves radiating out from the ganglia were cut, and all three ganglia were removed. The ganglia were taken out to minimize spontaneous activity and oxygen consumption by thoracic muscles other than the one that was to be stimulated. The waxy surface was scraped off the cuticle overlying the metathoracic tergosternal muscle of one side. Holes were punched through this cuticle with a fine insect pin, and a pair of silver wires, 50 μm in diameter, were inserted for stimulating electrodes. A hook made from a segment of a fine insect pin was fixed to the cuticle of the muscle insertion. The hook and the stimulating electrodes were held in place with cyanoacrylate contact cement. The hook served as an attachment point for the ergometer. After the electrodes and hook were in place, a ring of cuticle was removed from around the muscle insertion so as to free the ventral end of the muscle. The muscle was moistened as needed with saline (composition given in Usherwood, 1968, pH adjusted to 7 with NaOH before use).

Throughout the preparation of the muscle care was taken to minimize damage to the tracheal system of the thorax through which oxygen reaches the muscles. To ensure adequate oxygen supply in our preparation, we inserted a cannula into the mesothoracic spiracle through which air was perfused. The mesothoracic spiracle and associated trachea most directly supply the tergosternal muscle (Weis-Fogh, 1964). The cannula was fixed in place with low-melting-point wax. Then the head and abdomen were cut off, and the thorax was fixed to a glass plate with rapid-setting epoxy cement.

Mechanical power output

The mechanical power output of the tergosternal muscle was determined using the work-loop technique, in which the muscle is subjected to periodic length change and stimulated phasically in the length cycle. The area of the loop formed by plotting muscle length against muscle force is the net mechanical work done per cycle (Josephson, 1985; Fig. 1). The work per cycle multiplied by the cycle frequency is the mechanical power output.

The ergometer consisted of a force transducer mounted on the end of a shaft attached to a servo-controlled puller motor (Ling 102A shaker). The force transducer was made from a pair of semiconductor strain gauges. A hook on the end of the transducer engaged the hook fixed to the insertion of the tergosternal muscle. The position of the ergometer was adjusted so that the muscle was aligned with the transducer, and the muscle was at its normal *in vivo* length as judged by the relative positions of the cuticle at the muscle insertion and surrounding structures.

The muscle was subjected to sinusoidal length change at 20 Hz, which is the normal wing stroke frequency for a locust the size of *S. americana* (Weis-Fogh, 1956). The muscle was stimulated with 0·5 ms shocks, usually with one shock per cycle. Values for muscle length and force were collected with an analog-to-digital converter. A total of 600 force–position pairs were collected for a single cycle and analysed with a microcomputer to determine the work output for that cycle.

A small thermistor inserted into the thorax near the muscle measured temperature and served as the sensor in a servo system that controlled the intensity of a microscope lamp whose beam was directed at the thorax. The servo system maintained the thoracic temperature at 30 °C.

When an insect muscle is stimulated through wires implanted in or near the muscle, the tension

evoked to a series of shocks of gradually increased intensity generally increases in a few discrete steps, corresponding to activation of individual motor units (e.g. Josephson, 1973). Although the stimulation is nominally direct, the muscle fibres apparently become activated by impulses initiated in the motoneurons to the muscle. With the tergosternal muscle there were two steps in

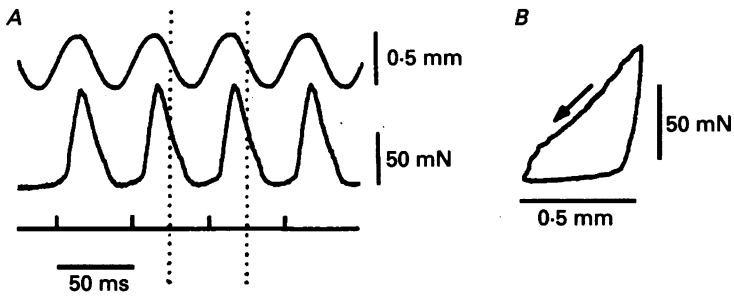

Fig. 1. Work cycles from the tergosternal muscle. *A*, the muscle was subjected to sinusoidal strain at 20 Hz (upper trace) and phasic stimulation (lower trace, one shock per cycle). The middle trace is muscle force. *B*, a plot of muscle force against length for the cycle in *A* indicated by dotted lines. The area of such loops, here 27 μJ, is the work per cycle. The amplitude of the sinusoidal length change and the timing of the stimulation were adjusted to maximize work output.

the amplitudes of isometric twitches recorded during a series of single shocks of gradually increased intensity from below threshold to well above threshold, indicating that this muscle contains only two motor units. Except where otherwise indicated, the stimulus intensity in efficiency measurements was just above threshold for the more sensitive of the two units. Higher intensities were not used because of the risk of activating nearby muscles which would have increased the oxygen consumption without an associated increase in the measured work output. Each preparation was observed carefully when the thresholds of the motor units were measured. A few preparations were discarded because the stimulus intensity required to activate the more sensitive of the tergosternal motor units was close to that at which neighbouring muscles began to contract.

The mechanical power output from a muscle undergoing sinusoidal length change at a given frequency varies with the amplitude of the imposed length change (the strain) and the time in the length cycle at which the muscle is stimulated (stimulus phase). We measure stimulus phase from the relationship between a fictive parameter, the expected time of peak muscle force had the contraction been isometric, and the imposed length cycle. A phase of 0 indicates that the expected time of peak force coincides with maximum muscle length. Positive phase (from 0 to 50%) indicates that the expected time of peak force is during muscle shortening; negative phase (0 to −50%) that the expected time of peak force is during muscle lengthening. The optimal muscle strain and the optimal stimulus phase were determined by systematically varying these parameters while measuring work output (see Josephson, 1985, for the search procedure). Strain and phase determinations were made using five cycles of imposed length change and muscle stimulation at 20 Hz. Trials were repeated once each 30 s. If not otherwise indicated, work and power were measured at optimum stimulus phase and muscle strain. During measurements of efficiency the muscle was stimulated at 20 Hz for 10, 20 or 30 s. Values for force and position over a cycle were collected, the work analysed, and the value stored each 1·2 s during these trials. Measurements of efficiency were repeated at 10 min intervals.

At the end of an experiment the muscle was fixed while still attached to the ergometer by superfusion with 70% ethanol. After storage for several days to several weeks in 70% ethanol, the preparation was dissected and the length of the experimental and control muscles were measured. The length of experimental muscles averaged 98% of the length of the contralateral control muscles (S.D. = 3%, $n = 48$), showing that the length chosen for the experimental muscle during

measurements was similar to the *in vivo* length. The muscle was rehydrated overnight in saline and weighed. Locust muscles lose about 18% of their mass when they are fixed in alcohol and later rehydrated (Malamud, 1989). Compensation was made for this mass loss in estimating the original wet weight of the muscle. The ratio of muscle mass and muscle length (assuming the density of the muscle to be 1×10^3 kg m^{-3}) gave muscle cross-sectional area.

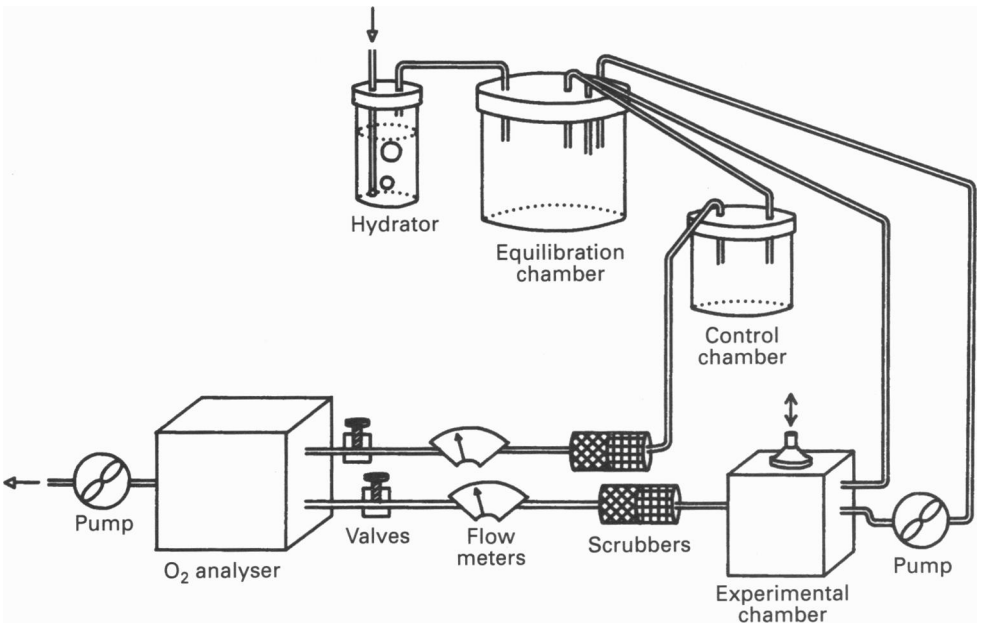

Fig. 2. The apparatus for measuring oxygen consumption. The preparation was sealed within the experimental chamber. The shaft of the ergometer, whose movement is indicated by the double arrow, entered the chamber through a flexible diaphragm. The air line through a pump into the experimental chamber provided tracheal perfusion. The scrubbers removed water vapour and CO_2.

Oxygen consumption

The glass plate to which the thorax was glued was mounted in the bottom of a small (18 ml) leucite chamber. One side wall and the top of the chamber were removable to allow introduction of and manipulation of the preparation. The side wall and top were subsequently attached to the rest of the chamber and sealed in place using bolts and silicone grease. The shaft of the ergometer entered the chamber through a flexible diaphragm. The stimulating electrodes, the leads from the force transducer, and the leads from the thermistor in the thorax were connected to the outside through airtight sockets built into the wall of the chamber. The tracheal cannula was connected to an external peristaltic pump through a tube in the chamber wall. The microscope light that heated the preparation shone through one wall of the chamber which was made of thin glass.

Hydrated air passed, in sequence, through a mixing chamber, the experimental chamber containing the preparation, a water scrubber (Drierite), a CO_2 scrubber (Ascarite), an oxygen detector, and finally the pump (Fig. 2). The air flow rate through the chamber, 47·5 ml min^{-1}, includes both that directly from the mixing chamber and that coming from the mixing chamber through the peristaltic pump and the tracheal cannula (1·3 ml min^{-1}). The pressure within the experimental chamber during measurements, as determined with a water-filled manometer, was -15 cmH_2O) ($= -15$ kPa).

The oxygen analyser (Ametek S-3A/II) was a differential instrument. The oxygen content of the air in the experimental pathway was compared with that in a similar pathway but in which the experimental chamber was replaced by an empty chamber of equivalent volume. In order to reduce noise in the output from the oxygen analyser, the signal was passed to the digital data-acquisition system through a second-order low-pass filter (time constant = 1 s) and sometimes the signal was subjected to digital averaging as well. The output of the oxygen analyser was sampled each time a work loop was analysed. The calibration of the oxygen analyser was frequently checked by injecting known amounts of oxygen into the airstream entering the experimental chamber. The resolution of the oxygen record after filtering was 10–20 parts per million.

Because of conduction times in the tubing and the scrubbers, and because of wash-out time from the experimental chamber, a change in the oxygen concentration measured by the oxygen analyser lagged well behind a change in the oxygen concentration in the experimental chamber initiated by oxygen injection into the inflow stream or, presumably, by a change in oxygen uptake rate by the preparation. The values of the conduction time and of the wash-out time were determined from the time course of the signal from the oxygen analyser in response to the rapid injection of a small amount of oxygen into the inflow line to the chamber. The conduction time (delay between oxygen injection and the first signal) was about 11 s. After this delay, the oxygen signal rose to a peak in 6–10 s, and then fell with a time course which was well approximated by a single exponential decay with a time constant of 16 s (Fig. 5*b*). A fixed delay of 11 s and a chamber wash-out time constant of 16 s were assumed in correcting measured changes in oxygen consumption for the lags introduced by transit and wash-out times. The procedure used to correct for the chamber wash-out time was that of Bartholomew, Vleck & Vleck (1981).

Measuring efficiency

The usual trial in efficiency measurements consisted of 30 s of continuous stimulation at 20 Hz, with optimal stimulus phase and muscle strain for work output. Efficiency was calculated as the ratio of the net work done during a stimulation period and the energy equivalent of the oxygen consumed above the resting level. To calculate the net work output, the stimulation period was divided into a number of time bins of equal length, each centered on a cycle for which force and position values were collected and for which the work output was calculated. The analysed cycle was taken as being representative of all cycles in the bin. The net work for each bin was taken as the work done on the middle cycle multiplied by the number of cycles in the bin, with appropriate allowance being made for bins with a reduced number of cycles at the onset and end of the stimulation period. The oxygen consumption associated with a work period was determined as the area beneath the curve relating the change in oxygen concentration and time (Fig. 5*A*), evaluated over the whole period of elevated oxygen consumption, multiplied by the volume flow rate through the chamber. Oxygen consumption was converted to energy using a work equivalence of 20·1 J (ml O_2)$^{-1}$, based on an assumed respiratory coefficient (RQ) of 0·82 (Krogh & Weis-Fogh, 1951) due to the mixed combustion of fat (19·7 J (ml O_2)$^{-1}$) and carbohydrate (20·9 J (ml O_2)$^{-1}$, Schmidt-Nielsen, 1975).

RESULTS

General properties of the muscle

Table 1 summarizes the mechanical properties of the tergosternal muscle from *S. americana*. Adult females in this species are larger and have larger muscles than males. The twitch time course, the area-specific tension and the mass-specific work output were similar in both sexes. The twitch force was somewhat lower but otherwise the contractile properties and the mechanical work output of the tergosternal muscle were similar to those found in flight muscles of other locusts (Mizisin & Ready, 1986; Mizisin & Josephson, 1987; Malamud, Mizisin & Josephson, 1988).

The two motor units of the muscle produced similar forces and had twitches with similar time courses (Fig. 3, Table 1). The force generated when both motor units

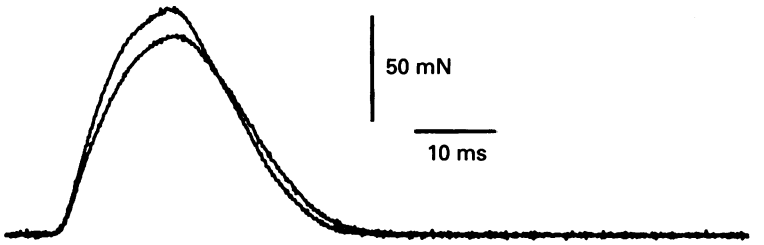

Fig. 3. Isometric twitches from the two motor units which comprised the tergosternal muscle, female locust. The units were differentially activated by reversing the polarity of stimuli through two implanted stimulating electrodes.

TABLE 1. General properties of the tergosternal muscle of *S. americana*

	Male	Female
Animal mass (g)	1·56 (0·19, 5)	3·02 (0·31, 5)
Thoracic mass (g)	0·58 (0·07, 5)	0·90 (0·04, 5)
Muscle size		
Mass (mg)	18·4 (1·9, 16)	27·4 (4·4, 30)
Length (mm)	7·84 (0·28, 16)	8·87 (0·51, 30)
Area (mm^2)	2·73 (0·21, 16)	3·59 (0·48, 30)
Twitch time course (ms)		
Rise time	14·7 (2·1, 11)	14·8 (1·7, 18)
Duration		
To 50% relaxation	25·8 (2·5, 11)	26·5 (2·6, 18)
To 90% relaxation	35·1 (3·4, 11)	36·2 (3·6, 18)
Twitch tension ($kN\ m^{-2}$)		
First unit	41·7 (9·9, 10)	41·0 (9·0, 18)
Both units	74·2 (12·6, 10)	71·8 (11·1, 18)
First*	29·2 (1·5, 2)	42·4 (8·7, 7)
Second	33·2 (4·7, 2)	35·5 (7·7, 7)
Both units	61·3 (6·5, 2)	71·1 (12·1, 7)
Work per cycle, first unit		
$J\ kg^{-1}$	1·11 (0·32, 16)	1·11 (0·33, 29)
Optimum phase (%)	20·8 (3·0, 16)	20·7 (2·3, 29)
Optimum strain (%)	6·0 (0·8, 16)	6·2 (0·73, 29)
Work per cycle,		
First unit/both units†	0·54 (0·05, 12)	0·53 (0·06, 16)

All values given as the mean, with S.D. and n in parentheses.

Values for contraction kinetics, twitch tension and work were obtained at 30 °C. Work was measured for the fourth cycle of a short burst at 20 Hz, with one stimulus per cycle, and at optimum strain and stimulus phase. 'First unit' refers to the motor unit with the lowest threshold of the two that make up the muscle. It is unlikely that the first unit was the same morphological motor unit in each preparation. The muscle area and mass used in calculating stress and specific work for the first unit were those of the whole muscle.

* This set of data refers to a set of preparations in which it was possible to stimulate reliably each of the two units separately, either by changing stimulus polarity or by using different pairs among three implanted stimulating electrodes.

† The work for both units was determined at that strain and stimulus phase determined to have been optimum for the first unit alone.

were excited simultaneously was quite similar to the sum of the forces produced by the two units individually, suggesting that there was no overlap in the muscle fibre populations excited by the two motor axons.

Work output per cycle was directly related to isometric twitch force (Fig. 4). Increasing the stimulus intensity so as to excite both motor units approximately

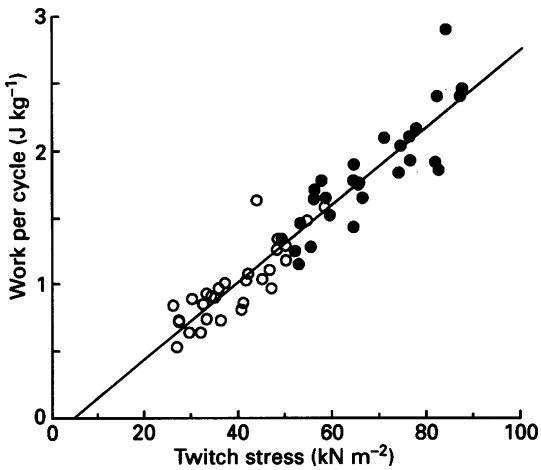

Fig. 4. Work output (fourth cycle of a short burst) as a function of the isometric twitch tension. Each open circle is from a separate preparation where one unit was activated. The filled circles are from most of the same preparations as the open circles, but for stimuli which activated both motor units in the muscles.

doubled the force output obtained with a single unit, and gave a proportional increase in work output (Table 1). The work per cycle for shocks which activated both units averaged 1·84 J kg^{-1} (S.D. = 0·39, n = 30), for a mechanical power output of 36·9 W kg^{-1}.

Muscle efficiency

With some muscles the work per cycle during efficiency trials was maximal at the beginning of the test period and declined thereafter, but with many preparations the work per cycle increased or decreased slightly over the first few seconds and then was reasonably constant (Figs 5*A* and 8).

A change in the oxygen content in the air reaching the oxygen analyser was first seen about half-way through the work period, and the change was greatest well after the termination of muscle stimulation (Fig. 5*A*). In order to better estimate the time course of oxygen consumption, the oxygen record was corrected for transit time between the experimental chamber and the oxygen analyser and for the exponential wash-out time of the chamber. The corrected oxygen consumption rose rapidly at the onset of stimulation to a plateau and began to fall shortly after the cessation of stimulation (Fig. 5*A*). Even after correcting for chamber and transit delay the change in oxygen concentration lagged behind the work output, especially at the cessation of muscle activity. The different time courses of oxygen consumption and

work output are due, at least in part, to equilibration time for gases between the animal's tracheal system and the experimental chamber, and to incomplete compensation for chamber delays. It is apparent in measures of calibration pulses of oxygen subjected to the same correction procedures that the compensation for

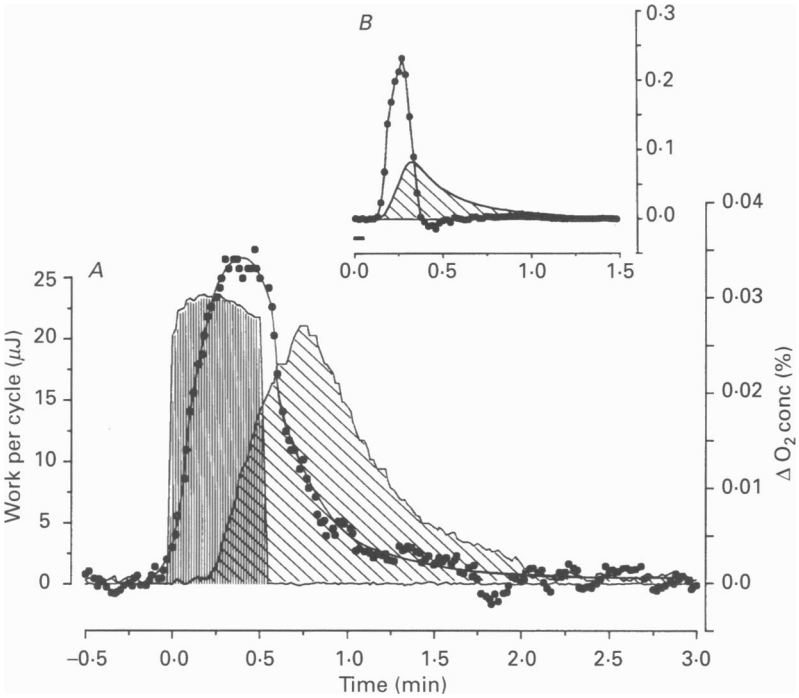

Fig. 5. *A*, muscle work (▥) and oxygen consumption (standard temperature and pressure) for 30 s of work output at 20 Hz, one stimulus per cycle. The measured changes in oxygen concentration (ΔO_2 conc, ▨) were corrected (●) for time delays using a fixed delay of 11 s for the transit time between the experimental chamber and the analyser, and an exponential wash-out time for the experimental chamber of 16 s. The data points were subjected to a seven-point sliding average before plotting them or correcting for delays. An upward deflection indicates a decrease in oxygen concentration. *B*, change in measured oxygen concentration following injection of a calibration pulse of oxygen (15 μl) into the experimental chamber (upward deflection indicates increase in oxygen concentration). The initial data points were subjected to a seven-point sliding average. In the corrected record the data were amended to compensate for the exponential wash-out time of the chamber. To facilitate comparison between uncorrected and corrected curves, no compensation was made for transit time. The approximate duration of the injection is indicated by the short horizontal bar.

chamber delay is imperfect (Fig. 5*B*). Changes in oxygen concentration, both corrected for chamber delay and uncorrected, fell to prestimulation levels less than 2 min after the end of stimulation, confirming expectations that flight muscle metabolism is aerobic and that no long-term oxygen debt is incurred during the activity periods (see also Krogh & Weis-Fogh, 1951).

Muscle efficiency, defined as the ratio of work output to metabolic energy input, ranged from 4 to 10% in different muscles (Fig. 6), with a mean value of 6·4% (S.D.

= 1·8%, $n = 46$). Efficiency did not vary with the strength of the muscle; muscles with low capacity for work output had a concomitantly reduced oxygen consumption (Figs 4 and 6).

In six preparations, three male and three female, efficiency was measured during 30 s trials both from the motor unit with the lowest threshold and, by increasing the stimulus intensity, from the two motor units simultaneously. The mean efficiency of the single motor unit was 6·2% (S.D. = 1·2), that for both units 6·4% (S.D. = 0·5), and in each individual preparation the efficiencies were similar with one and with both motor units. Apparently the two motor units have similar efficiencies.

Efficiency and duration of activity period

Work output was generally stable throughout test periods, with little sign of fatigue (Figs 5*A* and 8). In order to determine whether efficiency was also stable over time, we measured efficiency during 10, 20 and 30 s trials. The experimental design was fully balanced. Results were obtained from six male muscles and six female muscles. Efficiencies were measured from each muscle at the three trial durations. With each muscle the three durations were presented twice, in two mirror-symmetrical sets (for example: 30, 20, 10, 10, 20, 30 s). The order of presentation of the three durations was systematically varied from preparation to preparation so that all possible orders were used for each sex.

The measured efficiency was essentially the same for each duration of stimulation period. The mean efficiencies for the 10, 20 and 30 s trials were: 5·85% (S.E.M. = 0·49), 6·21% (0·47), and 6·16% (0·48). Thus efficiency seems independent of time of activity, at least over the range of work periods considered.

Work and efficiency with two stimuli per cycle

It has been proposed that changing the number of action potentials per cycle is a mechanism used to control power output by insects with synchronous muscles (e.g. Wilson & Weis-Fogh, 1962; Kammer, 1985). It has been shown directly that multiple stimuli per cycle can increase the power output above that available with single stimuli in locust and tettigoniid wing muscles (Josephson, 1985; Mizisin & Josephson, 1987). It should be noted that giving multiple stimuli increases the force per contraction above that for single stimuli, but it also increases the duration of the contraction. In locust muscles at 30 °C and 20 Hz, the twitch duration for single shocks is about the same as the half-cycle duration. Even with optimal stimulus timing, the increased contraction duration with multiple stimuli results in increased force in the lengthening half-cycle and increased work expended in re-extending the muscle, which partially and sometimes completely negates the increased work output associated with the increased force during shortening (Stevenson & Josephson, 1990).

The following experiments were done to determine whether the increased work output expected with multiple stimulation is at the expense of muscle efficiency. In six preparations, three male and three female, the optimum stimulus phase and muscle strain for work output were determined for the fourth cycle of short bursts using both single stimuli per cycle and paired stimuli separated by 6 ms. In an earlier study 6 ms was found to be the optimum interstimulus interval for power output

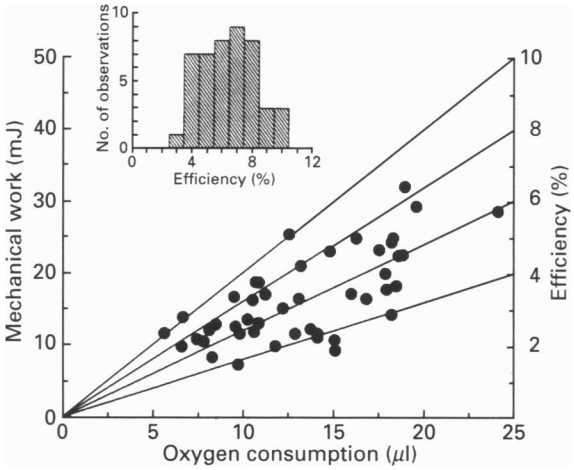

Fig. 6. Oxygen consumption and mechanical work output. Each point is from a different preparation and represents the value for the first trial with 30 s of activity at 20 Hz (one stimulus per cycle) for that preparation. Diagonal lines indicate efficiency, based on the assumption that 1 ml of O_2 is equivalent to 20·1 J. The histogram is the distribution of efficiency values for this data.

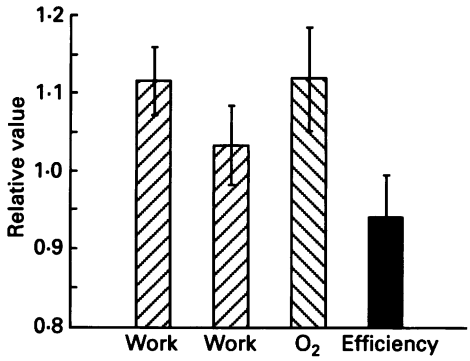

Fig. 7. Work, oxygen consumption and efficiency with two stimuli per cycle (interstimulus interval = 6 ms) relative to those obtained with a single stimulus per cycle. The first bar is the work on a single cycle, which was the fourth of a short burst. The other bars are for 30 s of activity. The error bars are two S.E.M.S.

from a locust muscle when using two stimuli per cycle (Mizisin & Josephson, 1987). The optimum strains, mean (S.D.), for one and two stimuli per cycle were 5·4% (0·6) and 6·2% (0·5%), and the optimum stimulus phases were 21% (2·3) and 18% (4·4%). The work per cycle with two stimuli was 13% greater (S.E.M. = 2%) than that with one stimulus per cycle (Fig. 7), which is a smaller enhancement than those found with the tergocoxal muscle of another locust species (20%, Mizisin & Josephson, 1987; 33% Malamud, Mizisin & Josephson, 1988).

After having determined the optimum strain and stimulus phase for one and two stimuli per cycle, the total work output and oxygen consumption at the optimum strain and phase were determined for 30 s activity periods in which trials with two

stimuli per cycle alternated with trials with a single stimulus per cycle. The work per cycle declined substantially over the course of a 30 s trial when using two stimuli per cycle, whereas it did not with one stimulus per cycle (Fig. 8). The work per cycle was consistently greater with two stimuli per cycle than with one early in the 30 s trial,

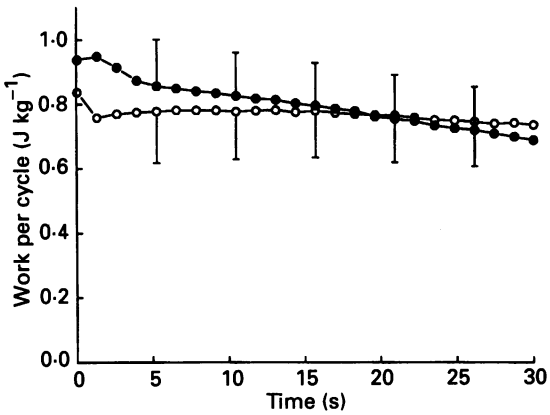

Fig. 8. The work per cycle during 30 s trials with one (○) and with two (●) stimuli per cycle. Vertical bars are standard deviations (shown in one direction only). The data are from six preparations. For each preparation the values for one stimulus per cycle were the averages of the work at a given time on the first and third trials of the series. The values for two stimuli per cycle were from a single trial, the second of the series, from each preparation.

but by the end of the trial the work with two stimuli per cycle was usually less than that with a single stimulus. The more rapid fatigue with two stimuli per cycle may be an experimental artifact, due to impaired tracheal function in the partially dissected preparations. But the results of Fig. 8, accepted at face value, suggest that multiple firing per cycle may enhance power output only for brief periods. Multiple firing may be useful in manoeuvring but not in steady flight. The efficiency with two stimuli per cycle averaged 93% (S.E.M. = 3%, $n = 6$) of that with one stimulus per cycle. The decline in efficiency with two stimuli per cycle, while not great, was statistically significant ($P < 5\%$, one-tailed t test on paired samples). The increased power output with two stimuli per cycle is at some cost in muscle efficiency.

Oxygen consumption during isometric contraction

The energy output of a muscle, measured as the heat produced plus the work done or by the metabolic changes which support energy output, is often greater when the muscle shortens against an external load and does work than when the muscle contracts isometrically (the Fenn effect; reviewed by Rall, 1982; Woledge, Curtin & Homsher, 1985). Oxygen consumption was compared during work cycles and during isometric contractions at the same frequency in order to determine whether there is a Fenn effect in the cycling locust muscle and if so, to measure its magnitude. Even during nominal isometric contraction there can be some work done by the muscle in shortening against the compliance of the transducer and the muscle attachments. The movement of markers placed on the end of the tergosternal muscle near the

transducer was measured with a dissecting microscope. The excursion of these markers during isometric twitch contractions at 20 Hz indicated that the shortening of the muscle against the transducer was generally less than 1 % of the muscle length. In order to reduce further possible contributions to oxygen consumption of work

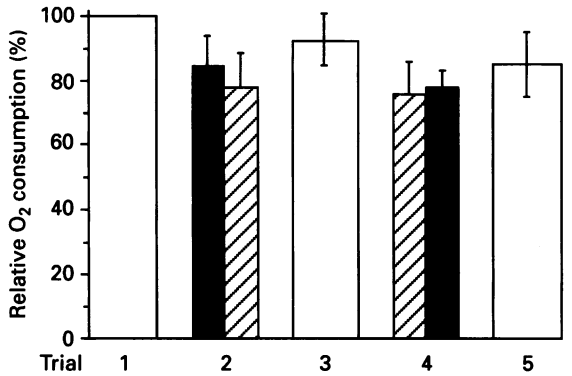

Fig. 9. Relative oxygen consumption during sequential 30 s trials with positive work output (□), isometric contraction (■), or negative work output (▨). Error bars are two S.E.M.S. See text for details.

done during shortening, oxygen consumption was also measured during negative work loops (work loops in which the timing of stimulation was such that the muscle was active during the lengthening portion of the cycle and inactive during shortening). The negative work loops were obtained by changing the phase of stimulation by one half-cycle from that which was optimal for work output. At the reversed phase the muscle was stimulated shortly before the onset of muscle lengthening.

The three conditions for which oxygen consumption was measured in this series were: (1) 30 s of positive work output at 20 Hz, optimum stimulus phase and optimum muscle strain; (2) 30 s of isometric contractions at 20 Hz; and (3) 30 s of negative work at 20 Hz, the strain that was optimum for positive work, and stimuli one half-cycle out of phase from the optimum for positive work. Trials were presented in the following order: (1) positive work, (2) isometric contraction (or negative work in half the preparations), (3) positive work, (4) negative work (or isometric contractions if the second trial was negative work), and (5) positive work. Six male and six female muscles were used. In three preparations from each sex the second trial was the set of isometric contractions while in the other three it was negative work. The net work done during negative work trials (mean $= -1{\cdot}14$ J kg^{-1} cycle^{-1}, S.E.M. $= 0{\cdot}12$, $n = 12$) was about twice the absolute amplitude and of opposite sign to the net work done by the muscle during positive work trials (mean, based on the average of the two positive work trials bracketing the negative work trial, $= 0{\cdot}64$ J kg^{-1} cycle^{-1}, S.E.M. $= 0{\cdot}08$, $n = 12$).

The oxygen consumption associated with positive work output was greater than that during isometric contractions, but the difference was not large (Fig. 9). In the data in Fig. 9, the oxygen consumption during the trials with isometric contraction averaged 85·6% (S.E.M. = 4·2) AND 88·9% (S.E.M. = 2·9) of the average of the

preceding and following trials with positive work. The oxygen consumption during trials with negative work was essentially the same as during isometric contraction. Apparently the extra energy costs of shortening and doing external work are not very large as compared to the isometric costs, which represent the energy needed to activate the muscle and to generate isometric force.

The data from this set of experiments also provides a measure of the stability of the flight muscle preparation. For the experiments of Fig. 9, the average work per cycle over a 30 s trial declined from 0·87 J kg^{-1} (S.E.M. = 0·08, $n = 12$) on the first trial to 0·61 J kg^{-1} (S.E.M. = 0·07) on the fifth trial; and the energy equivalent of the oxygen consumed per cycle declined from 14·1 J kg^{-1} (S.E.M. = 0·7) to 11·6 J kg^{-1} (S.E.M. = 0·6). These mass-specific values are based on the mass of the whole muscle, which is expected to be about twice that of the single motor unit actually active. Thus the work per twitch declined by about 31% and the oxygen consumption by about 17% over the 30 mins and approximately 1800 muscle contractions that occurred between the first and the fifth trials.

DISCUSSION

Overall efficiency

The efficiency of the locust flight muscle, about 6% (Fig. 6), is substantially lower than values typically reported for frog and mammalian muscles (15–30% for initial plus recovery processes; Gibbs & Gibson, 1972; Wendt & Gibbs, 1973; Stainsby, Gladden, Barclay & Wilson, 1980; Heglund & Cavagna, 1987; De Haan, Van Ingen Schenau, Ettema, Huijing & Lodder, 1989). However, the low efficiency measured for the locust muscle is consistent with some recent estimates of the efficiency of insect flight muscles based on metabolic rate during hovering or flight and either the calculated aerodynamic and inertial energy costs of remaining aloft or directly measured muscle power output (Ellington, 1985; Casey & Ellington, 1989; Stevenson & Josephson, 1990). The question arises as to why the efficiency of insect flight muscle is so low. Part of the reason for low efficiency may lie in high activation costs associated with the moderately high frequency (20 Hz) at which the muscle operates.

Activation costs

The activation cost for muscle activity, that energy expended in turning muscle on and off, should increase with operating frequency (see, for example, Goldspink, 1977; Heglund & Taylor, 1988) if a fixed amount of calcium is released from internal stores or enters across the surface membrane each time the muscle is activated and is resequestered or expelled after each activation. Further, for a muscle with a given force–velocity characteristic, the maximum mechanical work done during the shortening phase of a cyclic contraction is inversely proportional to operating frequency or nearly so (Josephson, 1989). As a first approximation, putting aside complexities introduced by the time course of stimulus-evoked muscle activation and the effects of this time course on the instantaneous power during shortening and on the work required to relengthen the muscle, the power output of a muscle, which is the product of work per cycle and cycle frequency, is expected to be roughly independent of operating frequency. The consequence of power output, which is not

a function of frequency, and activation costs, which are proportional to frequency, is efficiency, which declines with frequency. It is estimated that calcium cycling costs account for 25–50 % of the energy input supporting contraction and work output of frog muscle (Kushmerick & Davies, 1969; Homsher & Kean, 1978). We are suggesting that the calcium cycling costs of the locust muscle may be substantially greater than those of frog muscle because of the higher frequency at which the locust muscle operates, and that the high activation costs contribute to the low efficiency of the locust muscle.

Activation costs alone may be too small to be the sole cause of low efficiency. For the forty-six preparations in Fig. 6, the total work output of the locust muscles over the 30 s test periods averaged 0·55 kJ (kg muscle)$^{-1}$, and the caloric equivalent of the oxygen consumed was 8·90 kJ kg^{-1} for an overall efficiency of 6·2 %. Assuming that the single unit from which work and oxygen was measured in these studies made up half of the total muscle (Table 1), and dividing by the 600 twitches in the test period, gives a work output of 1·83 J (kg active muscle)$^{-1}$ cycle^{-1} and a metabolic input of 29·7 J (kg active muscle)$^{-1}$ cycle^{-1}. If the efficiency of the contractile process alone were 20 %, the metabolic input to support the work itself would be 9·2 J kg^{-1} cycle^{-1}, leaving 20·5 J kg^{-1} cycle^{-1} for the activation cost. This activation cost is substantially greater than that measured from vertebrate muscles (5 to 12 J kg^{-1} twitch^{-1} for frog muscle including both initial costs and measured or estimated recovery costs (Homsher, Mommaerts, Ricchiuti & Wallner, 1972; Smith, 1972; Rall, 1978; Burchfield & Rall, 1985; see also Kushmerick, Larson & Davies, 1969); and 1–4 J kg^{-1} twitch $^{-1}$ for several bird and mammalian muscles (Gibbs & Gibson, 1972; Rall & Schottelius, 1973; Wendt & Gibbs, 1973)). Either the activation costs are considerably greater in the locust muscle than in the vertebrate muscles studied, or the efficiency of the contractile machinery itself is lower.

Elastic energy storage

As mentioned above, one of the uncertainties in previous estimations of the efficiency of insect flight muscle has been the extent to which the kinetic energy of the moving wings is stored elastically as the wing decelerates at the end of its stroke. Ellington (1985) calculated the efficiency of insect flight muscle to be 5–8 % if there is perfect elastic storage of energy, and 12–29 % if there is no elastic storage (see also Casey & Ellington, 1989). The efficiency measured directly for the locust muscle, about 6 %, is that predicted for the case with good elastic storage, suggesting that there is indeed substantial storage of elastic energy in the insect flight system.

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