J. exp. Biol. 108, 377–392 (1984)
Printed in Great Britain © The Company of Biologists Limited 1984

THE SPECIFIC POWER OUTPUT OF AEROBIC MUSCLE, RELATED TO THE POWER DENSITY OF MITOCHONDRIA

BY C. J. PENNYCUICK
Department of Zoology, University of Bristol
AND MARCIO A. REZENDE
Department of Zoology, University of Bristol and Fundacão Parque Zoológico de São Paulo, Brazil

Accepted 14 July 1983

SUMMARY

A simple theory is proposed to account for the quantity of mitochondria present in aerobic muscles. Attention is restricted to muscles adapted to operate aerobically at a well-defined 'operating frequency'. For this special case, it is shown that the volume ratio of mitochondria to myofibrils should depend on the power density of mitochondria, and the operating frequency, but not on the mechanical properties of the myofibrils. If the underlying assumptions are valid, this would mean that the specific power output of such muscles could be determined by examination of electron micrographs.

We provisionally estimate that the inverse power density of mitochondria, in flight muscles running at a high temperature, is in the range $1{\cdot}0\,10^{-6}$ to $1{\cdot}3\,10^{-6}\,m^3\,W^{-1}$, that is, that a little over 1 ml of mitochondria is required to sustain 1 W of mechanical power output. On this basis, a muscle with equal volumes of mitochondria and myofibrils should be able to deliver a specific power of about $430\,W\,kg^{-1}$, at an operating frequency around 40 Hz for non-fibrillar, or 230 Hz for fibrillar muscle. The limiting specific power should be twice this level in either case, i.e. about $860\,W\,kg^{-1}$.

It is predicted that a survey of flight muscles should yield a straight-line relationship between wing-beat frequency and the volume ratio of mitochondria to myofibrils, in a set of muscles of the same general type. It is not known whether lack of exercise, either on a long-term or short-term basis is likely to affect this. As a preliminary to such a survey, we have examined the pectoralis muscles of domesticated quail, and a wild house sparrow. Both showed a high level of variability in the mitochondria: myofibril ratio, but this may be due, at least in part, to sampling artifacts caused by the shape of mitochondrial arrays. The quail showed distinct populations of aerobic and non-aerobic fibres, apparently identical to those described in wild birds with similar flight requirements by George & Berger (1966), but the quantity of mitochondria in the aerobic fibres was less than half that expected, by comparison with other species.

Present address: Department of Biology, University of Miami, P.O. Box 249118, Coral Gables, Florida 124, U.S.A.

Key words: Muscle, power, mitochondria.

INTRODUCTION

Estimates of the mechanical power available from muscles are often required for locomotion models, but the information is difficult to get directly. Very indirect estimates can be obtained by measuring the whole animal's oxygen consumption, assigning a fraction of it to locomotion, and making an assumption about conversion efficiency. In this paper we try a different type of assumption. It is assumed that a muscle used for some well-defined locomotor activity is adapted to work under identifiable mechanical conditions, that this adaptation is manifested in structural characteristics which can be measured in electron micrographs of muscle sections, and that these measurements in turn can be used to estimate the power output.

The power output of a muscle is the rate at which it does mechanical work. For many purposes specific power output is a more convenient variable, and this may be expressed in two forms. Mass-specific and volume-specific power output are respectively the rates of doing work per unit mass and per unit volume of muscle. Whichever form is used, it has been known for many years that specific power output is strongly dependent on the frequency of contraction (Hill, 1950). More recently Weis-Fogh & Alexander (1977) have investigated the limits of specific power output, based on the known mechanical properties of various muscles, and the requirements for converting and supplying energy to the contractile proteins. The latter function is performed by mitochondria, which are embedded in aerobic muscle fibres. Weis-Fogh & Alexander's analysis is extremely general, in that it allows the muscle to perform work under a somewhat wider range of mechanical conditions than might be expected to prevail during cruising locomotor activity. We propose that if we restrict the discussion to a narrow range of conditions, some simple principles may emerge, which, whilst not absolutely general, are nevertheless valid for a large and important class of locomotor activities.

THEORETICAL BACKGROUND

Simplified muscle model and definitions

The schematic muscle shown in Fig. 1 has an extended length L, and shortens through a distance ΔL. The 'active strain', r, is defined as

$$r = \Delta L / L . \tag{1}$$

If the time taken for shortening is t, then the 'strain rate' is defined as r/t. The cross-sectional area, A, is supposed to remain constant during shortening. The muscle exerts a tension force F, and a stress s, where

$$s = F / A . \tag{2}$$

Specific work

The work done, Q, during the contraction is $F\Delta L$, which may also be expressed, from equations 1 and 2, as

$$Q = sA \times rL . \tag{3}$$

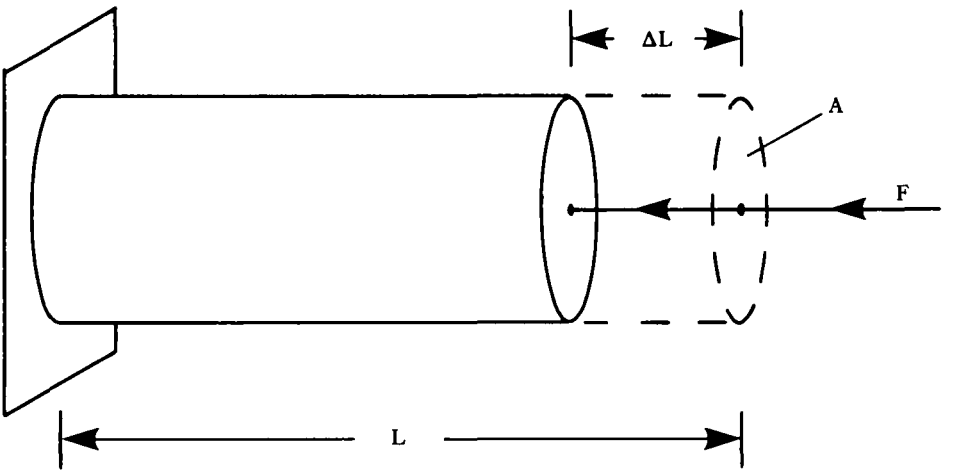

Fig. 1. A schematic muscle is attached to the skeleton at left, exerts a force F, and shortens through a distance ΔL, starting from an extended length L. Its cross-sectional area is A.

The product of A and L is also the volume, v, of the extended muscle. The 'volume-specific work', Q_v, is therefore

$$Q_v = Q/v = sr, \tag{4}$$

that is, the stress times the active strain is the work done per unit volume of muscle. This can be converted into the mass-specific work, Q_m, by dividing by the density, ϱ, of the muscle:

$$Q_m = Q_v/\varrho = sr/\varrho. \tag{5}$$

Operating frequency

Our interest originates with the flight muscles of birds which are capable of sustained, aerobic flight. Our next simplifying assumption is that selection for such a relatively well-defined activity results in the muscles being adapted to work at a particular contraction frequency, which we call the 'operating frequency'. For example, a migrating bird has to fly within a restricted speed range if it is to maximize the distance flown on a given supply of fuel, and this speed restriction in turn defines one particular flapping frequency. This frequency is determined by the aerodynamical and inertial properties of the wings and body, not by the properties of the muscles. The 'operating frequency' is defined by the structure of the animal, and the type of activity which has to be sustained. The properties of the muscles then have to be matched in turn to this frequency. We propose that the adaptation consists of adjusting the maximum strain rate of which the myofibrils are capable.

Maximum strain rate (intrinsic speed)

Restriction to a particular operating frequency, and a particular type of work cycle, also defines the time taken by the muscle to shorten, and thus the strain rate whilst shortening is in progress. The strain rate in turn determines the stress which the

myofibrils are able to exert. The relationship between stress and strain rate was discovered by Hill (1938), in the slightly different form of a relationship between force and velocity. Hill found that in frog skeletal muscle, the speed of shortening, V, was related to the force exerted, F, by the hyperbolic equation

$$(F + a)V = b(F_o - F), \tag{6}$$

where F_o is the maximum force which the muscle can exert in an isometric contraction, and a and b are also constants. b has the dimensions of velocity, and a has those of force.

The instantaneous power, P, developed whilst the muscle is shortening, is equal to the product of the force times the velocity, that is

$$P = FV = bF(F_o - F)/(F + a). \tag{7}$$

It can be shown by differentiating this with respect to F that the maximum power is obtained when

$$F = (a^2 + aF_o)^{0.5} - a. \tag{8}$$

The force constant, a, is related (not equal) to the mechanical efficiency of the muscle, and likely to have similar values for muscles of the same general type. Hill found that in frog skeletal muscle, a was about equal to $F_o/4$, and this value can be taken as representative for vertebrate skeletal muscle. In that case, equation 8 shows that the maximum power is obtained at a force equal to $0{\cdot}31F_o$. The power is, of course, zero at both ends of the curve, when either the force or the speed is zero.

When applying his force-velocity relationship to considering locomotion, Hill (1950) re-expressed it in the form shown in the upper graph of Fig. 2, in which strain rate (instead of velocity) is plotted against stress (instead of force). This does not affect the shape of the graph, but changes the scales of the two axes in a way which facilitates comparison between different muscles. If the abscissa is understood to represent the stress across the myofibrils (as opposed to that averaged across the whole muscle), a common scale can be used for all muscles of the same general type, irrespective of their size. The righthand end of the scale is defined by the maximum isometric stress which can be exerted when the strain rate is constrained to be zero. Weis-Fogh & Alexander (1977) estimate the maximum isometric stress which can be exerted by the myofibrils of vertebrate skeletal muscles to be in the range 250–400 kN m^{-2}, and we use a value of 300 kN m^{-2} to define the scale of Fig. 2.

The curves A and B of Fig. 2 compare two hypothetical muscles, characterized as 'fast' and 'slow'. They differ in the maximum strain rate (also known as the 'intrinsic speed'), that is, the strain rate at which each shortens against zero stress. In isometric contraction, both fast and slow muscles exert the same maximum stress. In the lower curve of Fig. 2, the ordinate has been converted into volume-specific power, by multiplying the strain rate by the stress. This instantaneous power is the rate of doing work whilst shortening is actually in progress. As noted above, it shows a rather flat peak at a stress (or force) about 31 % of the maximum, and drops to zero when the strain rate is either at its maximum value, or zero.

It was suggested above that the strain rate at which the muscle is permitted to contract during cruising locomotion is fixed by factors external to the muscle. Th

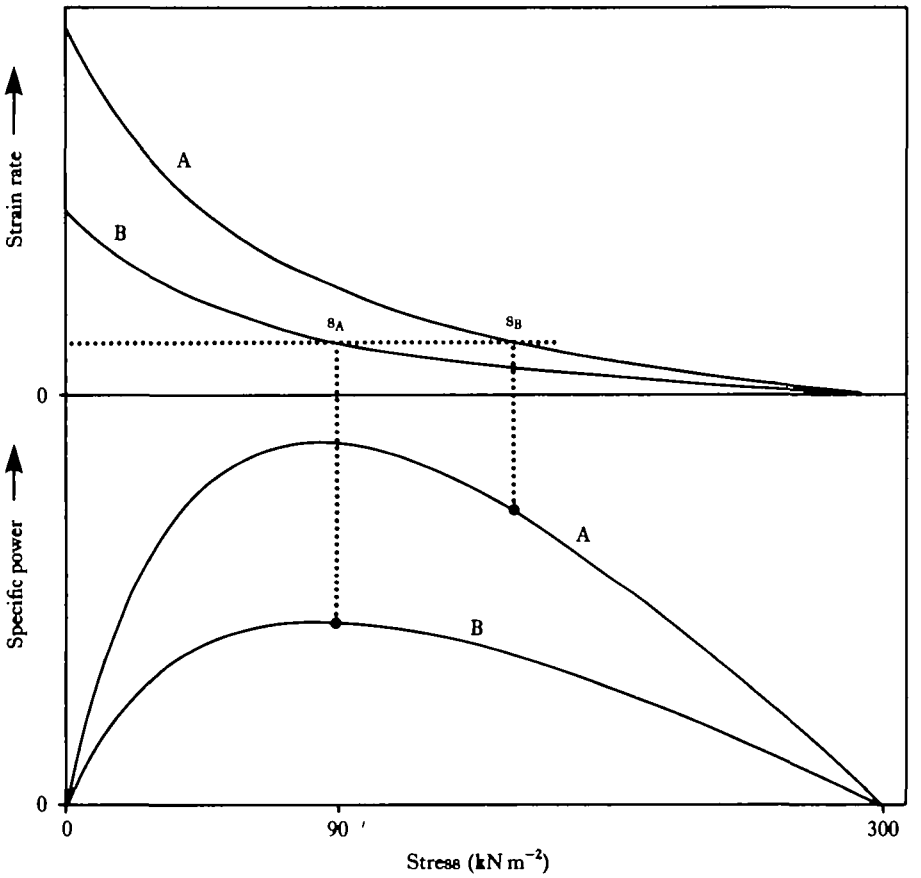

Fig. 2. Curves of strain rate and instantaneous specific power output, as functions of stress, calculated from Hill's equation, for (A) a fast and (B) a slow muscle.

is represented by the horizontal dotted line across the upper graphs of Fig. 2. This particular value of strain rate results in the slower muscle (B) exerting the stress s_B, which corresponds to its maximum power (lower graph). At the same strain rate, the faster muscle (A) exerts a higher stress, s_A, and therefore produces more power than the slower muscle, even though the operating point is displaced well to the right of its peak power in the lower graph. The curve of instantaneous power *versus* stress therefore does not define an optimum value for the maximum strain rate. If such a value exists, it must be set by other considerations, most probably those of efficiency.

Efficiency as a function of stress

Hill (1964) defined 'efficiency' during contraction as the ratio of work done to the sum of work plus heat produced. He showed that efficiency can be deduced, as a function of stress, from his equation relating strain rate to stress. The maximum efficiency is obtained at about 40–50 % of the isometric stress in frog striated muscle. If the stress is either zero, or equal to the isometric stress, no work is done, and the efficiency (like the instantaneous power) is zero.

We are primarily concerned with muscles specialized for prolonged aerobic activity

(as in bird migration) rather than maximal exertion. Such muscles would seem likely to be adapted to maximize efficiency. We therefore assume that the maximum strain rate is so adjusted that the muscles exert about half the isometric stress during cruising activity. They would then be operating slightly below their maximum power, like the faster muscle in Fig. 2, perhaps giving a reserve of power which could be exerted anaerobically for short periods.

Representative stress and strain values

In the subsequent argument we need estimates of stress across the myofibrils, and active strain, during shortening in cruising locomotion. For vertebrate striated muscle, we take the stress to be 150 kN m^{-2} (50 % of the probable maximum, as above). We assume in effect that the stress during lengthening is zero in vertebrate striated muscles. This would certainly not be a good assumption for insect fibrillar muscles. Here we need the difference in stress between shortening and lengthening. This is difficult to estimate, but Weis-Fogh & Alexander (1977) suggest 200 kN m^{-2} as a reasonable figure.

A figure of 0·25 is often quoted for the active strain of vertebrate striated muscle, from experiments with muscle preparations removed from the animal. In an animal with a rigid skeleton, the actual strain permitted in locomotion is determined by the geometry of the joints and muscle attachments. In principle it can be measured by manipulation of joints, but no survey of actual strains seems to have been carried out. Gordon, Huxley & Julian (1966) related the isometric tension of a striated muscle to the position of the sliding filaments, over a wide range of lengths, and their analysis suggests that a strain of 0·25 would take the muscle into regions where either the ends of the actin filaments overlap, or not all the cross-bridges are attached. With virtually no empirical information to go on, we take a value of 0·15 as an estimate of the actual strain in non-fibrillar muscles, remembering that this refers to prolonged cruising locomotion rather than maximal exertion. Estimates of the active strain in insect fibrillar muscles range from 1–3 %, and we take 0·02 as a representative figure. The stress and strain in non-fibrillar insect muscles, such as the flight muscles of Orthoptera and Lepidoptera, are assumed to be similar to those in vertebrate muscle. The values used for later calculations are summarized in Table 1.

Sustained power

The power of interest in sustained locomotion is not the instantaneous power, considered above, but the average power (P) over a number of complete cycles of contraction and relaxation. It is equal to the work done in each contraction, times the contraction frequency, which is here taken to be the operating frequency, as defined above:

Table 1. *Stress and strain values assumed during shortening*

Muscle type	Stress (kN m^{-2})	Strain
Vertebrate striated	150	0·15
Insect fibrillar	200	0·02

$$P = Qf. \tag{9}$$

Similarly the volume-specific and mass-specific power are obtained by multiplying the respective specific work by the operating frequency:

$$P_v = Q_v f = srf, \tag{10}$$

and

$$P_m = Q_m f = srf/\varrho. \tag{11}$$

Equations 10 and 11 suggest that (1) the specific power output of the muscles of a series of different animals of the same general type should be directly proportional to their operating frequencies, and (2) that at any particular operating frequency, the specific power of insect fibrillar muscle is much less than that of vertebrate striated muscle, because of its lower specific work. These conclusions would be correct if muscles could produce sustained power without containing mitochondria, but have to be modified when the mitochondria are taken into consideration.

Effect of mitochondria

We now consider a muscle which contains two components, a volume v_c of myofibrils and a volume v_t of mitochondria. Together, these make up the whole volume, v, of the fibre:

$$v = v_c + v_t. \tag{12}$$

We suppose that the volume of mitochondria required is directly proportional to the mechanical power output, P:

$$v_t = kP, \tag{13}$$

where k is a constant with the dimensions of volume/power. k is the volume of mitochondria required to sustain unit mechanical power output, and is referred to as the 'inverse power density of mitochondria'.

We now specify that s is the stress exerted across the myofibrils (not across the whole muscle), in which case

$$P = srfv_c, \tag{14}$$

as before, and, from equation 13,

$$v_t = ksrfv_c. \tag{15}$$

We can now find the volume-specific power output for the whole muscle, which is

$$\begin{aligned} P_v &= P/(v_c + v_t) \\ &= srfv_c/(v_c + ksrfv_c) \\ &= srf/(1 + ksrf). \end{aligned} \tag{16}$$

The mass-specific form of this is

$$P_m = srf/\varrho(1 + ksrf). \tag{17}$$

Comparison of this result with equation 11 shows that the effect of adding mitochondria is to divide the specific power output of the myofibrils alone by a factor

(1+ksrf). Our subsequent figures indicate that at very low operating frequencies (below about 10 Hz), this factor is negligibly greater than 1, so that equation 11 can be considered to apply to the whole muscle. However, at very high frequencies, ksrf is much greater than 1, and in that case equation 17 approximates to

$$P_m = 1/\varrho k . \tag{18}$$

At very high frequencies the specific power output tends asymptotically towards an upper limit determined by the properties of mitochondria, and independent of the mechanical properties of the myofibrils.

Ratio of myofibrils to mitochondria

If we define q as the ratio of v_t to v_c, then equation 15 can be written

$$q = ksrf . \tag{19}$$

Substituting this in equation 17 yields

$$P_m = q/\varrho k(1 + q) . \tag{20}$$

Equation 20 does not involve the mechanical properties of the myofibrils at all. It implies that if an estimate can be obtained for the value of k, the specific power output of any aerobic muscle can be determined from an electron micrograph. For example, muscles are known in both insects and hummingbirds which contain approximately equal volumes of myofibrils and mitochondria. In that case, q = 1, and equation 20 becomes

$$P_m = 1/2\varrho k , \tag{21}$$

that is, a muscle with this characteristic should have a specific power equal to half the ultimate value given by equation 18, regardless of the stress or strain generated by the myofibrils.

Equation 20 offers a very simple method for estimating the specific power output of any aerobic muscle, provided an estimate is to hand for k, the inverse power density of mitochondria. It is possible that k may vary in different species, in relation to differences in the size and internal structure of their mitochondria, the conversion efficiency of the contractile machinery or the type of fuel being oxidized. However, as direct measurements of k do not seem practicable at the moment, we shall begin by assuming that k has a constant value for all muscles working at a particular operating temperature (discussed further below). This is a starting hypothesis, which may have to be modified in the light of later evidence. Caution is also needed in relation to our underlying assumption, that the ratio of mitochondria to myofibrils truly reflects the specific power output, at the operating frequency observed in cruising locomotion. We cannot be certain whether the quantity of mitochondria in muscles varies in a short-term manner in response to exercise training. It is possible, for instance, that the quantity of mitochondria in the flight muscles of a migratory bird might change at different times in the migration cycle. If that were so, then only samples taken during the actual migratory period could be relied on to conform to equation 20. On the other hand the volume fraction of mitochondria may be genetically fixed, and not subject to short-term variations, in which case measurements on

the muscles of zoo birds could be used to make deductions about muscle performance of the species in the wild.

MATERIALS AND METHODS

Preparation of specimens

Samples of pectoralis muscle were taken from Japanese quail (*Coturnix coturnix*), reared in a laboratory culture, and a wild house sparrow (*Passer domesticus*), using a 'Tru-Cut' biopsy needle, manufactured by Travenol Laboratories Inc. The quail were either under deep halothane/nitrous oxide anaesthesia, from which they were not allowed to recover, or freshly killed by a blow on the head. The sparrow was sampled immediately after a fatal collision with a car.

The muscle samples were fixed for 2 h at room temperature in 2·5 % glutaraldehyde, buffered to pH 7·5 in 0·05 M-sodium cacodylate containing 2·5 % sucrose. This was followed by three 20-s rinses in cacodylate/sucrose buffer, then 1 h in 1 % osmium tetroxide in buffer.

After embedding the specimens in Araldite, sections 50–90 nm thick were cut, using an LKB 4800 microtome with a glass knife, and stained with uranyl acetate (20 min), then with alkaline lead citrate (10 min). They were examined with a Philips 300 electron microscope. The eventual magnification of the prints (on Ilfospeed resin-coated paper), from which measurements were made, varied from 6200 to 7800 diameters.

Area measurements

The object of measurements on the electron micrographs was to determine the relative volumes occupied by various cell components. The volumes had to be inferred from measurements of areas on the prints. Measurements were made on sections cut approximately transverse to the long axis of the fibres. Diameters of fibres were measured directly, the smallest diameter being chosen in fibres of elliptical shape. Cross-sectional areas were measured with the aid of a 'Bit-Pad' digitizing tablet, interfaced to a Commodore PET 4032 computer. The following cross-sectional areas were measured on each fibre:

1. The whole fibre.
2. Total for all mitochondria in the fibre.
3. Total for cell components other than mitochondria or myofibrils.

The area of myofibrils was obtained by subtracting the areas of mitochondria and 'other' components from that of the whole fibre.

RESULTS

Volume fraction of nuclei

Areas in the third category above proved to be very variable in the pectoralis muscle. In the quail, most fibre cross-sections contained less than 1 % of 'other' components, but a few contained much more, one fibre (out of 265) reaching 28 %. The larger fractions occurred when the section happened to pass through a nucleus.

On average, 2·8% of each fibre's cross-sectional area was occupied by 'other' components, mainly nuclei and sarcoplasmic reticulum. This was taken as an estimate of the volume fraction of the fibres occupied by these components. Nuclei and sarcoplasmic reticulum were somewhat more prominent in the supracoracoideus, but we confine attention here to the pectoralis.

Arrangement of mitochondria

In longitudinal section the mitochondria were seen to be arranged in linear arrays parallel to the myofibrils. These arrays were cigar-shaped, and only about 1·5–7 μm long, much shorter than the myofibrils, which appeared to extend for several millimetres. Thus transverse sections (Fig. 3) showed a large number of myofibrils of relatively uniform diameter, and a smaller number of mitochondrial arrays, of more variable diameter, depending whether the section had passed through the middle or the end of the array. The number of mitochondrial arrays sectioned in any particular fibre was also quite variable. These sampling effects gave rise to a level of variability of the area fraction occupied by mitochondria, as seen in different fibre sections, which did not necessarily reflect a similar variability in the volume fraction of mitochondria in whole fibres. In general we expect that the volume fraction of mitochondria should depend strongly on the operating frequency, and therefore should not vary greatly between different aerobic fibres of the same muscle.

Aerobic and anaerobic fibres

In Fig. 4 (quail) the diameter of each fibre is plotted against the area fraction of mitochondria. It can be seen that the points form two clusters. Fibres with many mitochondria were small, with diameters up to 20 μm, whereas those with few mitochondria mostly had diameters in the range 20–70 μm (one at 85 μm). Such a division into two distinct fibre types was described by George & Berger (1966) in the pectoralis of birds which they classified as 'Group 3', including the domestic pigeon (*Columba livia*), but not the domestic fowl (*Gallus gallus*). They classified the latter as 'Group 1', having mostly large fibres with few mitochondria, and only a few with variable amounts of mitochondria. In view of the domesticated origin of our quail, it is perhaps surprising that their muscles apparently resemble those of flying birds so closely. The actual quantity of mitochondria, however, is anomalous (below).

We characterize the two fibre types as 'aerobic', to the right of the long dotted line in Fig. 4, and 'anaerobic' to the left. In the sparrow the large fibres with few mitochondria were not seen, and all the fibres were considered 'aerobic'. In the analysis which follows, we are concerned with the mitochondrial content of the aerobic fibres only. The mean area fraction occupied by mitochondria was 14·3% in the quail, and 29·9% in the sparrow.

For our purposes, it is more convenient to consider the ratio of the area of mitochondria to that of myofibrils, as shown in the histograms of Fig. 5. The mean area ratios of mitochondria to myofibrils from Fig. 5 are 0·18 and 0·45 for the quail and sparrow respectively, and we take these as estimates of the volume ratios.

Wingbeat frequency

Wingbeat frequencies were taken from the literature, as follows. Bruderer &

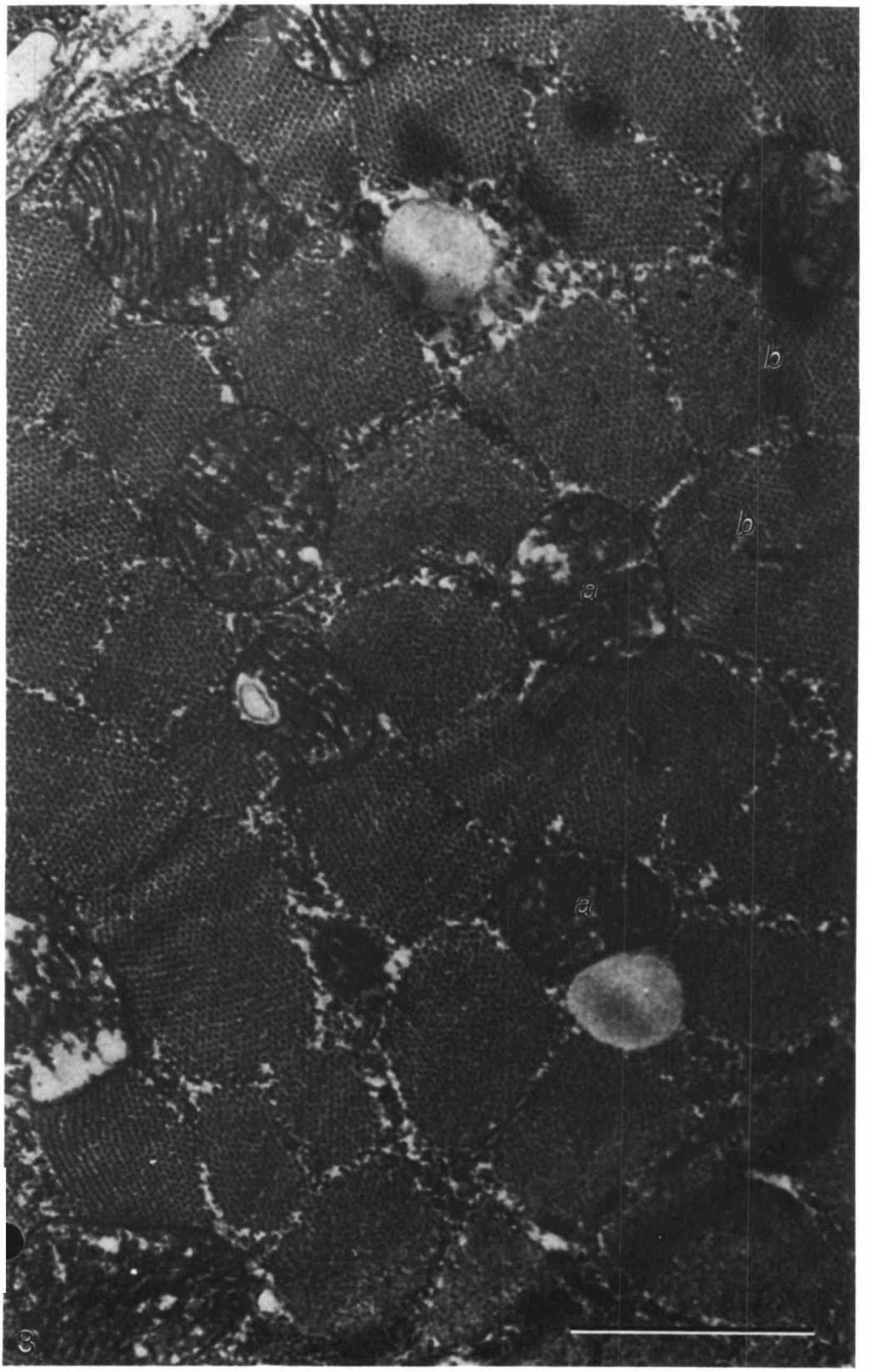

Fig. 3. Transverse section of quail pectoralis muscle, showing mitochondria (*a*) and myofibrils (*b*).

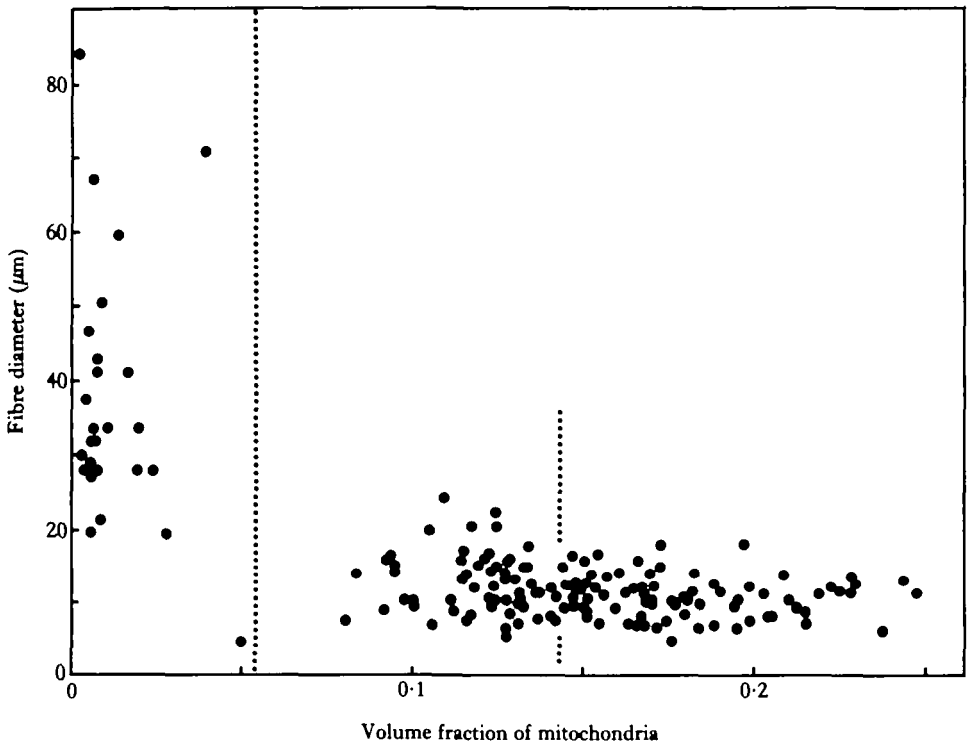

Fig. 4. Fibre diameter plotted against volume fraction of mitochondria for the quail pectoralis, showing distinction between anaerobic (left) and aerobic fibres. The short vertical dotted line marks the mean volume fraction of mitochondria for the aerobic fibres (14·3 %).

Jacquat (1972) obtained radar observations of wingbeat frequency in migrating quail, with a mean of 17·8 Hz. Pye (1981) observed a London sparrow, also by radar, flying at 10 m s^{-1} in 'bounding' flight, with bursts of flapping lasting about half the total time. Within each burst of flapping, the frequency was 19–21 Hz. By taking a value of 20 Hz we assume, in effect, that a migrating sparrow would flap continuously at this frequency.

DISCUSSION

Inverse power density of mitochondria

We can now attempt to estimate k, the inverse power density of mitochondria, from equation 19. Estimates are listed in Table 2 for five species, for which values are available for the operating frequency, and the ratio of mitochondria to myofibrils. The stress and strain are taken from Table 1. The locust's flight muscles, being non-fibrillar, are assumed to be mechanically similar to those of vertebrates, whereas the fly *Phormia* of course has fibrillar muscles.

With the exception of that for the quail, the estimates of k are in the region of 10^{-6} m^3 W^{-1} or a little over, which would mean that about 1 ml of mitochondria is required to sustain 1 W of mechanical power output. The estimate for the quail is less

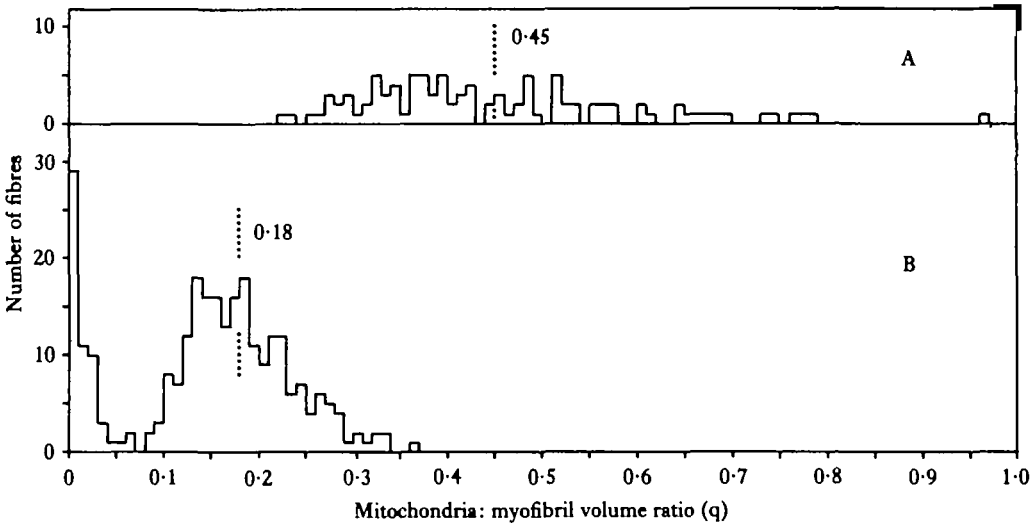

Fig. 5. Distribution of q, the mitochondria: myofibril volume ratio, estimated from areas in transverse sections. The mean marked for the quail (B) refers to the aerobic fibres only. Anaerobic fibres were not seen in the sparrow (A).

Table 2. *Estimates of k for various species*

Species	f (Hz)	q	k ($m^3 W^{-1}$)	Source
Coturnix coturnix	18	0·18	$4·4 \times 10^{-7}$	1
Passer domesticus	20	0·45	$1·0 \times 10^{-6}$	1
Schistocerca gregaria	19	0·43	$1·0 \times 10^{-6}$	2
Amazilia fimbriata	35	1·0	$1·3 \times 10^{-6}$	2
Phormia regina	164	0·73	$1·1 \times 10^{-6}$	3

Data sources: 1. Present study. 2. Weis-Fogh & Alexander (1977). 3. Levenbook & Williams (1956). Stress and strain assumed as in Table 1.

than half of this, which means that less mitochondria were present in its muscles than in the other species, relative to the operating frequency. Most probably this implies that these domesticated birds do not have enough mitochondria in their muscles to sustain aerobic flight.

In qualitative terms, as noted above, the quail's pectoralis resembles those of birds such as pigeons, which are capable of sustained cruising flight, and also of strenuous 'sprint' manoeuvres such as steep upward take-offs. These characteristics must have been retained from migratory ancestors through many generations of captivity. The western race of quail has been known to migrate since Old Testament times (Numbers 11,31), and was said more recently by Moreau (1961) to migrate non-stop across the Sahara. The presence of aerobic fibres is understandable in this context, and our observations are anomalous only in respect of the actual quantity of mitochondria present in them.

The question remains open as to whether the volume fraction of mitochondria in the muscles of an individual bird can be adjusted on a short-term basis in response to exercise training. It is possible, for instance, that this could be achieved by rapid changes in the volume of individual mitochondria, without any change in their number

In that case, it is conceivable that laboratory quail could be trained, by exercise, to be capable of prolonged aerobic flight, even after centuries of domestication.

Predicted mass-specific power output

Fig. 6 shows plots of mass-specific power output calculated from equation 17, taking $k = 1{\cdot}1\times10^{-6}\,m^3\,W^{-1}$ as a representative value from Table 2. The two curves shown, for vertebrate striated and insect fibrillar muscle, are calculated with different values of stress and strain, as listed in Table 1. No great confidence can be placed in these assumed values, but it certainly is true that the stress-strain product is much less for insect fibrillar than for vertebrate striated, or insect non-fibrillar muscle. This results in each curve starting off from zero frequency with a slope equal to sr/ϱ, which is much steeper for the vertebrate than for the insect muscle. At around 40 Hz the vertebrate muscle reaches the level at which the volume of mitochondria equals that of myofibrils ($429\,W\,kg^{-1}$), whereas the fibrillar muscle reaches this point at about 230 Hz. Both muscles approach an asymptote of $858\,W\,kg^{-1}$ ($1/\varrho k$) at very high frequencies, but the muscle would consist mostly of mitochondria long before this level is reached.

Fig. 7 shows the mass-specific power for any muscle, as a function of the volume ratio of mitochondria to myofibrils. This is calculated from equation 20, which, as noted above, requires a value for k, but not for the stress or strain. With our assumed value for k ($1{\cdot}1\times10^{-6}\,m^3\,W^{-1}$), the specific power is $429\,W\,kg^{-1}$ when the volume of mitochondria is equal to that of myofibrils, irrespective of whether the muscle is fibrillar or otherwise.

With the values used, the vertebrate muscle reaches a specific power around $400\,W\,kg^{-1}$, at a frequency around 35 Hz, with nearly equal volumes of mitochondria and myofibrils. At this frequency the fibrillar muscle would produce only about $115\,W\,kg^{-1}$, but as the operating frequency is increased, its performance rapidly catches up with that of the vertebrate muscle.

It seems to be unusual for the volume of mitochondria greatly to exceed that of myofibrils in any muscle. According to equation 19, a given value of q corresponds to a particular value of the product of strain and frequency, and hence to a particular value of strain rate. Weis-Fogh & Alexander (1977) suggested that an upper limit to strain rate is inherent in the contractile mechanism itself, in which case it would be fortuitous that the upper limit of q seems to be about 1. On the other hand, it may be that too great a preponderance of mitochondria leads to difficulty in conveying ATP to the myofibrils. If that were so, small insects, needing wing beat frequencies much over 300 Hz, would actually have to restrict the mechanical properties of their myofibrils, in order to keep the specific power output down. The small strain of fibrillar muscles might then be seen, not as an unavoidable limitation of this type of muscle, but as an adaptation, serving to keep the volume of mitochondria within reasonable bounds.

Operating temperature

So far it has been assumed that a single value of k will apply to any mitochondria. It remains to be determined whether mitochondria of different sizes, and with different internal morphology, have similar power densities when operating at the same

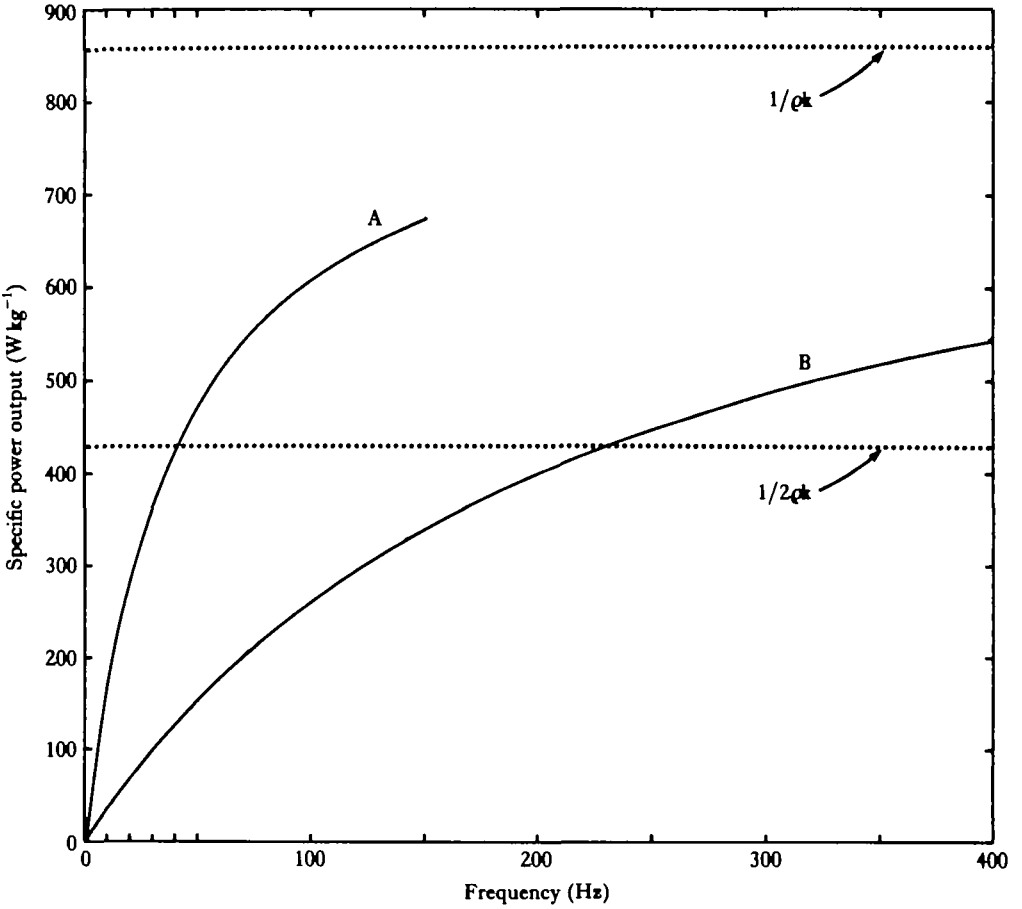

Fig. 6. Estimated mass-specific power output plotted as a function of operating frequency for (A) non-fibrillar, and (B) fibrillar muscle, from equation 17. The respective values for stress and strain are taken from Table 1, and k, the inverse power density of mitochondria, is taken to be $1{\cdot}1\times10^{-6}$ m^3 W^{-1}. The upper dotted line is the asymptote for both curves, and the lower one corresponds to q = 1, that is, equal volumes of mitochondria and myofibrils.

temperature. Be that as it may, the power density of a given mitochondrion will inevitably increase with temperature over a range of physiologically significant temperatures. k, the inverse power density, is expected to decrease with temperature, that is, less mitochondria are required to sustain a given mechanical power output at a high than at a low temperature. This must be an important reason, if not the main reason, why it is advantageous for active animals to maintain a high body temperature. Birds and mammals in general maintain their whole bodies at a high temperature, whereas many other animals maintain aerobic locomotor muscles at a higher temperature than the rest of the body. This is true of the flight muscles of many, if not all, flying insects (Heinrich, 1979) and the aerobic portion of the myotome muscles of certain pelagic fishes noted for sustained fast swimming (Carey, Teal, Kanwisher & Lawson, 1971). These include tunnies (*Thunnus, Euthynnus*) and some lamnid sharks (*Isurus, Lamna*).

Adapting the maximum strain rate, so that the muscle shortens at a particular stress

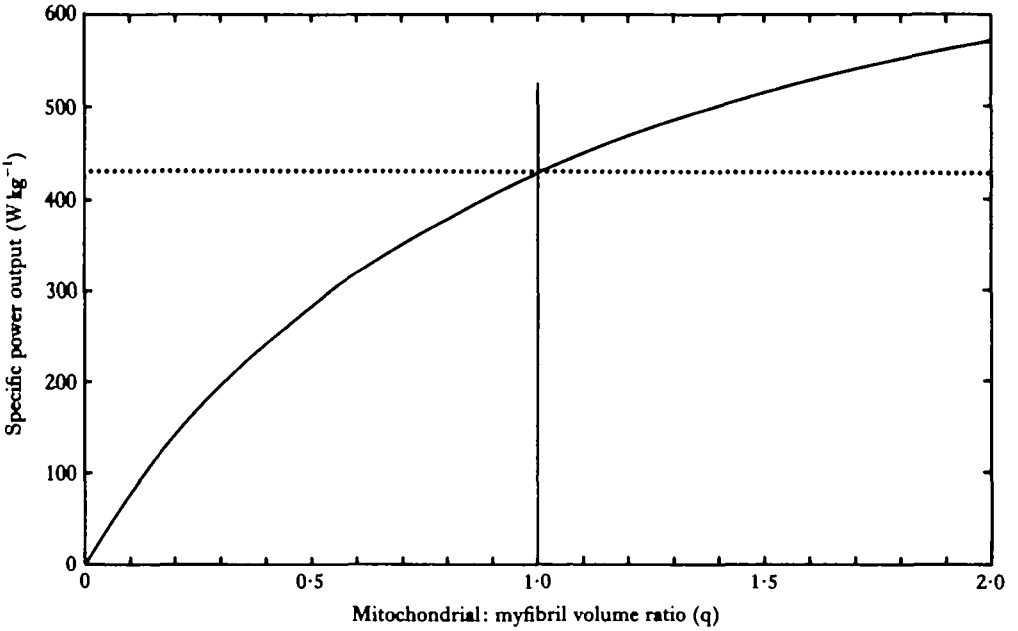

Fig. 7. Estimated specific power output as a function of q, the mitochondria: myofibril volume ratio, calculated from equation 20. This curve is independent of the mechanical properties of the myofibrils.

(above) requires that the operating temperature be fixed and predictable, but not necessarily high. The advantage of maintaining the highest possible temperature is that this minimizes the volume of mitochondria needed to sustain the required mechanical power output.

Collection of empirical data

The validity of the assumptions underlying equation 20 is not easy to establish, and will have to be investigated progressively. In the first place, equation 19 predicts that if the volume ratio of mitochondria to myofibrils is plotted against operating frequency, for a set of muscles with similar stress and strain, then a straight line should result, whose slope is equal to ksr. Two separate straight lines should result for fibrillar and non-fibrillar muscles. If the value of k is indeed the same for both, the difference of slope should be attributable to differences in stress and strain, of which the latter can be estimated by investigating the geometry of the wing articulation and muscle attachments. The biopsy technique should allow the effect of exercise training to be investigated in individual animals such as homing pigeons. The effect, if there is one, should take the form of deviations from the line of animals with reduced amounts of mitochondria in their muscles.

This research was supported in part by a research fellowship held by one of us (MAR) from the Research Foundation of Sao Paulo State (FAPESP), for whose support we are most grateful. We are also indebted to Dr Jeremy Rayner for helpful comments on a preliminary draft of the manuscript.

REFERENCES

BRUDERER, B. & JACQUAT, B. (1972). Zur Bestimmung von Flugelschlagfrequenzen tag- und nachtziehender Vogelarten mit Radar. *Orn. Beob.* **69**, 189–206.

CAREY, F. G., TEAL, J. M., KANWISHER, J. W. & LAWSON, K. D. (1971). Warm-blooded fish. *Am. Zool.* **11**, 135–143.

GEORGE, J. C. & BERGER, A. J. (1966). *Avian Myology*. New York: Academic Press.

HEINRICH, B. (1979). *Bumblebee Economics*. Cambridge, Mass: Harvard University Press.

GORDON, A. M., HUXLEY, A. F. & JULIAN, F. J. (1966). The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *J. Physiol., Lond.* **184**, 170–192.

HILL, A. V. (1938). The heat of shortening and the dynamic constants of muscle. *Proc. R. Soc.* B **126**, 136–195.

HILL, A. V. (1950). The dimensions of animals and their muscular dynamics. *Sci. Progr.* **38**, 209–230.

HILL, A. V. (1964). The efficiency of mechanical power development during muscular shortening and its relation to load. *Proc. R. Soc.* B **159**, 319–324.

LEVENBOOK, L. & WILLIAMS, C. M. (1956). Mitochondria in the flight muscles of insects. III. Mitochondrial cytochrome c in relation to the aging and wing beat frequency of flies. *J. gen. Physiol.* **39**, 497–512.

MOREAU, R. E. (1961). Problems of Mediterranean – Saharan migration. *Ibis* **103a**, 373–427.

PYE, J. D. (1981). Echolocation for flight guidance and a radar technique applicable to flight analysis. *Symp. zool. Soc. Lond.* **48**, 199–218.

WEIS-FOGH, T. & ALEXANDER, R. MCN. (1977). The sustained power output from striated muscle. In *Scale Effects in Animal Locomotion*, (ed. T. J. Pedley), pp. 511–525. London: Academic Press.