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OXYGEN AND THE UPPER LIMITS TO ANIMAL DESIGN AND PERFORMANCE

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Summary

Mass-specific rates of aerobic metabolism ($\dot{V}_{O_2}/M_b$) scale in inverse proportion to body mass (M_b). Thus, small hummingbirds display the highest $\dot{V}_{O_2}/M_b$ known among vertebrates. Among all animals, higher $\dot{V}_{O_2}/M_b$ values are known only in flying insects. The high body-mass-specific rates of metabolism seen in hummingbirds are made possible by high lung O_2 diffusing capacities, cardiac outputs, ratios of capillary surface area to muscle fiber surface area, mitochondrial volume densities, cristae surface densities and concentrations of enzymes involved in energy metabolism. Current evidence from control analyses of O_2 transport through the respiratory and cardiovascular systems and of metabolic fluxes through pathways of energy metabolism indicates shared control of maximum flux rates among multiple steps (i.e. the absence of single rate-limiting steps). This supports the suggestion that functional capacities at each step in linear pathways or processes are matched to each other, and provides an explanation for why the up-regulation of functional capacities has occurred at virtually all steps in the evolution of the smallest vertebrate homeotherms. Flying insects make use of a tracheal system for O_2 transport and, like hummingbirds, possess a highly up-regulated biochemical machinery for substrate oxidation. Studies of hummingbirds and honeybees reveal closer matches between biochemical flux capacities and maximum physiological flux rates than in animals capable of lower maximum $\dot{V}_{O_2}/M_b$. It is proposed that the upper limits to functional capacities set the upper limit to $\dot{V}_{O_2}/M_b$. This upper limit to aerobic metabolic rate may contribute, along with other factors, towards establishing the lower limit to vertebrate homeotherm size.

Key words: oxygen, performance, exercise, flight, hummingbird, metabolic rate, body mass, mitochondria, insect.

Introduction

A biochemist might rationalize the use of mitochondrial oxidative phosphorylation by animals that achieve the highest body-mass-specific metabolic rates ($\dot{V}_{O_2}/M_b$) on the basis of the high yield of ATP per mole of substrate oxidized, the high rates of ATP synthesis possible and the exquisite control mechanisms that allow oscillations of up to 2–3 orders of magnitude between basal and maximal rates. However, the oxidation of 1 mole of glucose or C_{16} fatty acid requires the consumption of 6 or 23 moles of O_2, respectively. Biochemical pathways have not evolved and do not function in animals in the absence of structural and functional context. Anatomists and physiologists would therefore superimpose upon the biochemist's conceptual framework the idea that the steady-state fluxes required for aerobic metabolism are achievable as a consequence of support systems and processes that supply O_2 and metabolic fuels to metabolically active organs. By adopting an integrative approach to the study of the energetics of exercise in animals, it has been possible to gain insights into how the design and function at the level of intracellular pathways of aerobic energy metabolism are related to the design and function of gas and fuel transport systems at higher levels of organization. Such an integrative approach is adopted here to analyze the design of organisms that achieve the highest mass-specific aerobic metabolic rates in the Animal Kingdom.

Small size, flight and energy metabolism

The inverse relationship between body mass and resting mass-specific metabolic rate is well known among comparative physiologists (Schmidt-Nielsen, 1984). This inverse relationship also holds for maximal aerobic body-mass-specific metabolic rates ($\dot{V}_{O_2max}/M_b$) (Taylor *et al.* 1989). Because of their small size and the energetic demands of flight, small hummingbirds display the highest known mass-specific aerobic metabolic rates among vertebrates (Suarez, 1992). Flying insects surpass hummingbirds and probably achieve the highest $\dot{V}_{O_2}/M_b$ of all animals (Sacktor, 1976). In my laboratory, hummingbirds and honeybees have served as useful models in studies of animal design and performance. The flight muscles of hummingbirds (Grinyer and George, 1969) and insects (Casey *et al.* 1992) consist of one fiber type. These

account for more than 90 % of whole-body O_2 consumption during steady-state exercise (Rothe and Nachtigall, 1989; Suarez, 1992). Such features make possible the estimation of O_2 and fuel flux rates into working muscles, as well as rates of substrate catabolism and enzyme function during flight.

Muscles are biological machines that convert chemical energy into mechanical work. At the contraction frequencies that characterize flight, ATP is hydrolyzed primarily by actomyosin ATPase, Ca^{2+}-ATPase and Na^+/K^+-ATPase (Homsher and Kean, 1978). In asynchronous insect flight muscles, such as those found in bees, an extensive sarcoplasmic reticulum is lacking, and Ca^{2+}-ATPase is thought to make a relatively minor contribution to ATP hydrolysis (Casey *et al.* 1992). Metabolic fuels are oxidized and O_2 is consumed by the muscle mitochondria as ATP is resynthesized at the same rate at which it is hydrolyzed. Unlike the locomotory muscles in larger mammals (Weber, 1997), hummingbird and insect flight muscles are both characterized by a high degree of reliance upon fuels originating from exogenous sources. Their highly aerobic metabolic organization requires that rates of mitochondrial O_2 consumption be matched by flux rates through the lungs, cardiovascular system, capillary walls in the case of hummingbirds (Fig. 1) and the tracheal system in the case of insects.

When a 4 g hummingbird hovers at a wingbeat frequency of 80 Hz, its $\dot{V}_{O_2}/M_b$ increases approximately 12-fold above basal resting values to approximately $40\,ml\,g^{-1}\,h^{-1}$ (Bartholomew and Lighton, 1986; Lasiewski, 1963), which is roughly 10 times higher than rates achieved by human athletes exercising at $\dot{V}_{O_2max}$ (e.g. Blomstrand *et al.* 1986). By flying hummingbirds in helium–O_2 mixtures less dense than air, it has been possible to induce short-term metabolic rates even higher than this (Chai *et al.* 1997). During 'normal' hovering, a steady-state activity hummingbirds engage in for the purpose of foraging on floral nectar, flight muscle O_2 consumption rates of approximately $2\,ml\,g^{-1}\,min^{-1}$ have been estimated, corresponding to rates of ATP turnover of close to $500\,\mu mol\,g^{-1}\,min^{-1}$ (Suarez, 1992; Suarez *et al.* 1990, 1991). Worker honeybees, hovering at wingbeat frequencies of 250 Hz, are even more impressive as their flight muscles consume $6\,ml\,O_2\,g^{-1}\,min^{-1}$ (Suarez *et al.* 1996). A single flying honeybee turns over (i.e. hydrolyzes and resynthesizes) 1.39×10^{15} molecules of ATP per wingbeat cycle!

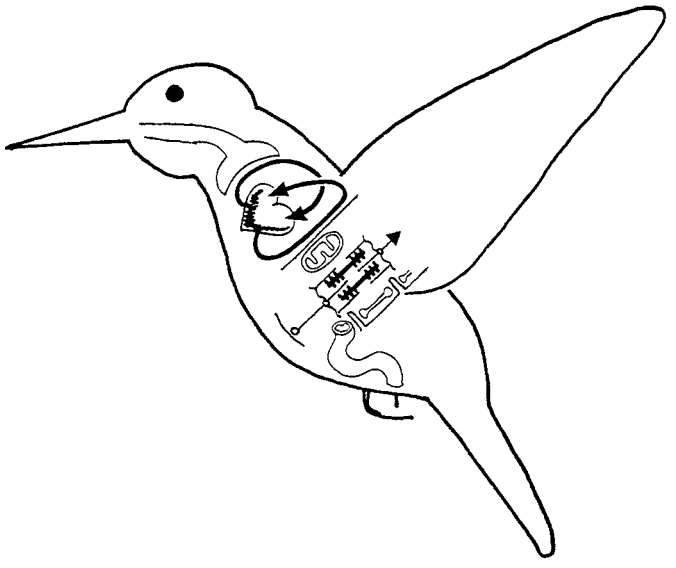

Fig. 1. Schematic diagram of a hummingbird in flight showing components relevant to the issues discussed in this paper. Hovering is made possible by large pectoral muscles, powered by abundant mitochondria, to which O_2 and metabolic fuels must be transported at high rates. These require high O_2 transport capacities through the respiratory and cardiovascular systems and high digestive capacities in the gut. Modified from a diagram of a frog (Weibel, 1985).

Up-regulating pathways for oxygen

Among vertebrates, skeletal muscle mitochondria are thought to be O_2-limited during exercise at $\dot{V}_{O_2max}$, and the control of O_2 flux rates is distributed or shared among all the convective and diffusive steps involved in O_2 transport from the external environment (Di Prampero, 1985; Wagner, 1996). The abandonment of the idea of single rate-limiting steps in favour of the concept of shared control of O_2 flux is supported both by theoretical calculations and by evidence demonstrating changes in $\dot{V}_{O_2max}$ resulting from perturbations experimentally induced at various steps. Given shared control of O_2 fluxes, it is not surprising to find up-regulation of O_2 flux capacities at multiple steps at all levels of organization in hummingbirds. Lung O_2-diffusing capacities, estimated by morphometric techniques, are estimated to be 8.5 times those of mammals of similar body mass (Dubach, 1981). Hummingbird hearts are twice as large as would be predicted on the basis of allometric relationships (Schmidt-Nielsen, 1984), while heart rates of approximately $1300\,min^{-1}$ have been measured (Lasiewski, 1964). Hematocrits, O_2 capacities and unloading efficiencies are high (Johansen *et al.* 1987), and pectoral muscle capillary volume densities are 2–6 times higher than in mammalian hindlimb muscles (Mathieu-Costello *et al.* 1992; Suarez *et al.* 1991).

Among the steps involved in the transport of O_2, the carrier-free zone between the red cell surface and the muscle membrane is thought to be a region of particularly high impedance (Honig *et al.* 1997). Thus, although intravascular P_{O_2} is high, intracellular (i.e. muscle fiber) P_{O_2} is thought to be much lower on the bases of theoretical calculations and estimates using various techniques. These have included microcryospectrophotometry (Gayeski *et al.* 1987) and ^{1}H-NMR spectroscopy (Richardson *et al.* 1995) to estimate the degree of O_2 saturation of myoglobin, as well as *in vivo* infrared spectroscopy to determine cytochrome redox state (Duhaylongsod *et al.* 1993). Hummingbird flight muscles consist of type II fibers with unusually small cross-sectional areas (Grinyer and George, 1969); each fiber in cross section is surrounded by an average of five capillaries (Mathieu-Costello *et al.* 1992) (Fig. 2). These result in large capillary

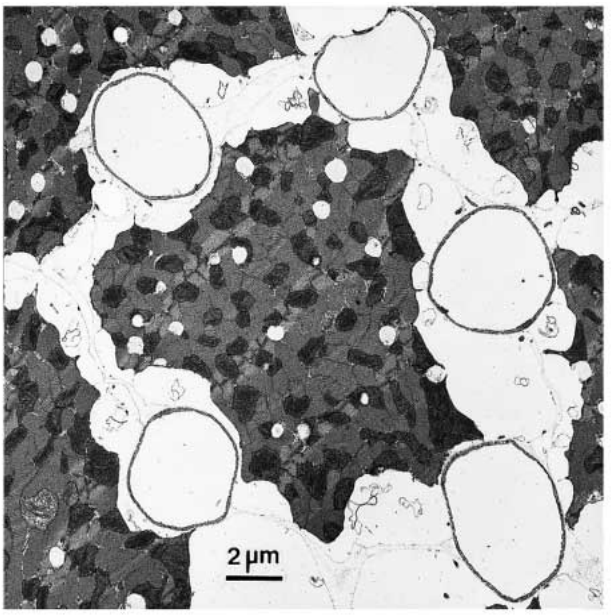

Fig. 2. Cross section of a muscle fiber from hummingbird (*Selasphorus rufus*) pectoralis, generously provided by O. Mathieu-Costello (University of California, San Diego). High mitochondrial volume density, small fiber cross-sectional area and the large number of capillaries (five may be seen here) result in high capacities for O_2 consumption. Scale bar, 2 µm.

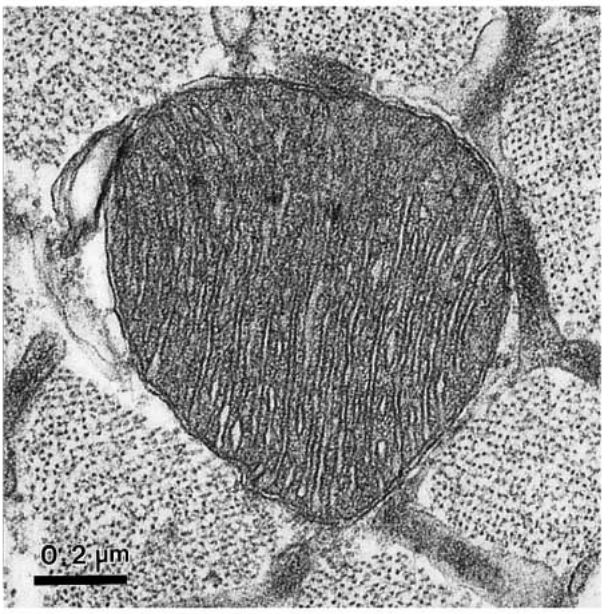

Fig. 3. High-magnification electron micrograph of a hummingbird (*Selasphorus rufus*) flight muscle mitochondrion showing cristae packed at a surface density of approximately $58\,m^2\,cm^{-3}$. This high cristae packing density partly accounts for the higher rates of O_2 consumption per unit mitochondrial volume during flight compared with the rates estimated in mammals exercising at $\dot{V}_{O_2max}$. It is apparent that a further increase in cristae surface density may result in insufficient matrix space required for Krebs cycle enzymes. This micrograph was also generously provided by O. Mathieu-Costello (University of California, San Diego). Scale bar, 0.2 µm.

surface areas relative to muscle fiber surface areas and, along with small diffusion distances between the sarcolemma and muscle mitochondria, result in significantly enhanced capacities for O_2 flux. In contrast with mammals, hummingbirds hovering under hyperoxic conditions do not increase $\dot{V}_{O_2max}$ values beyond those measured under normoxic conditions (Chai *et al.* 1996). This supports the suggestion of higher capacities for O_2 flux in hummingbirds than in mammals.

Myoglobin, which is thought to function as an important carrier of oxygen at high intracellular O_2 flux rates, occurs at high concentrations in hummingbird flight muscles (Johansen *et al.* 1987). Mitochondrial volume densities are approximately 35 %, which is among the highest known for vertebrate locomotory muscles (Suarez, 1992; Suarez *et al.* 1991). A substantially greater fraction of these mitochondria are localized under the sarcolemma than in mammalian skeletal muscles, further lessening intracellular diffusion distances for O_2. It has been suggested that this clustering of mitochondria adjacent to capillaries may result in further enhancement of capacities for O_2 flux (Mainwood and Rakusan, 1982). The mitochondrial cristae, where electron transport, O_2 consumption by cytochrome *c* oxidase and ATP synthesis *via* oxidative phosphorylation take place, occur at a surface density (surface area per unit volume) of $58\,m^2\,cm^{-3}$ (Suarez *et al.* 1991) (Fig. 3). This is between 1.5 and three times greater than the range of cristae surface densities found in mammalian skeletal muscles. Mitochondrial oxidative capacities in hummingbird flight muscles are therefore enhanced through more mitochondria and more inner membrane per mitochondrion.

Up-regulating pathways for substrate oxidation

In a historic deviation from the traditional concept of single rate-limiting steps in biochemical systems, metabolic control theory proposed, and metabolic control analysis has demonstrated, that the control of carbon flux is shared by multiple enzyme-catalyzed and membrane-carrier-mediated steps in pathways (Kacser and Burns, 1973; Fell, 1992). In recent years, much progress has been made in putting metabolic control theory into practice in the study of the regulation of muscle energy metabolism. In a landmark study, Kashiwaya *et al.* (1994) showed that the control of glucose utilization by isolated, perfused rat hearts is shared by the glucose transporter and various enzyme-catalyzed reactions in glycolysis. The relative degree of control exerted by several steps was shown to change by providing insulin or an alternative source of acetylCoA. In chemically skinned rat soleus, control analysis has been used to quantify the degree of control over O_2 consumption exerted by actomyosin ATPase as well as the mitochondrial reactions and transport processes involved in oxidative phosphorylation. Consistent with the idea that rates of ATP hydrolysis determine rates of ATP synthesis,

a flux control coefficient of 0.5 was estimated for actomyosin ATPase, while the rest of the control coefficients (adding up to a value of approximately 1) were found to be distributed among the mitochondrial reactions (Wisniewski *et al.* 1995). *In vivo*, muscles are characterized by their ability to undergo dramatic changes in metabolic flux rates with little change in the concentrations of pathway intermediates (Fell and Thomas, 1995; Thomas and Fell, 1996). Theoretical calculations and experimental manipulation of the level of expression of genes encoding single enzymes (e.g. Schaaff *et al.* 1989) indicate that changing the enzyme activities at single steps cannot account for such large changes in flux, leading to the proposal of multi-site modulation of metabolism (Fell and Thomas, 1995; Thomas and Fell, 1996). Given such distribution of control, one would expect that evolutionary up-regulation of flux capacities would be achieved not by the up-regulation of single (supposedly 'rate-limiting') enzymes or transporters, but rather through a concerted up-regulation of many reactions and transport steps in biochemical pathways. Consistent with this expectation, coordinated regulation of the expression of genes coding for glycolytic enzymes has, in fact, been observed (e.g. Robin *et al.* 1984; Webster, 1987).

V_{max} values (equal to $[E]\times k_{cat}$, where $[E]$ is enzyme concentration and k_{cat} is catalytic efficiency) serve as appropriate measures of flux capacities through pathways (Newsholme and Crabtree, 1986; Newsholme *et al.* 1980; Suarez, 1996). It has been recognized for decades that tissues capable of sustaining high rates of metabolic flux possess high enzyme V_{max} values throughout the relevant pathways. Accordingly, hummingbird (Suarez *et al.* 1986, 1990) and insect (Suarez *et al.* 1996) flight muscles possess high V_{max} values for many of the enzymes involved in the pathways of energy metabolism. An important issue from a mechanistic perspective is how homologous enzymes function in muscles across species: at what fractional velocities (v/V_{max}) do they work *in vivo* during exercise? From the perspective of evolutionary design, one may ask how capacities are related to maximum physiological loads; are these closely matched or do pathways possess 'too much' enzyme?

Enzymes catalyzing near-equilibrium reactions are typically found at V_{max} values 2–3 orders of magnitude higher than net forward flux rates (v) *in vivo* (Gitomer and Veech, 1988; Veech *et al.* 1969). However, it has been known at least from the time of Haldane's work on the subject (Haldane, 1930) that net forward flux at near-equilibrium reactions is possible only if V_{max} values greatly exceed v. Honeybee flight muscles possess V_{max} values for the enzyme phosphoglucoisomerase (PGI) that are approximately 20-fold greater than the rate of glycolysis during flight (Suarez *et al.* 1996). We have recently shown (Staples and Suarez, 1997) that these V_{max} values are very close (within 5 %) to what can be predicted using the Haldane equation, given the intracellular conditions in which the enzyme functions, its affinities for substrate and product, and the net glycolytic flux required for flight. These findings provide evidence in support of the biological relevance of enzyme kinetic parameters as well as the near-equilibrium nature of the PGI reaction during flight in honeybee flight muscles. Although it is claimed that such close matches between capacities and maximum physiological loads are unlikely to be the outcome of natural selection (Dudley and Gans, 1991; Garland and Huey, 1987), honeybees possess, in Diamond's words (Diamond, 1991), 'enough, but not too much' PGI.

Using data available from the literature as well as our own, we examined the relationships between flux rates (v) *in vivo* and V_{max} in various muscle types at the hexokinase, glycogen phosphorylase and phosphofructokinase steps, all nonequilibrium reactions in glycolysis (Suarez *et al.* 1997). The pattern that emerged reveals that, in species where maximum flux rates are relatively low, enzymes operate at very low v/V_{max}, whereas in muscles that achieve the highest flux rates, close matches between v and V_{max} are observed. This pattern implies, first, that homologous enzymes operate at higher fractional velocities in high-flux muscles and, second, that excess capacities are large at the low-flux end of the range but become smaller or even non-existent in species whose muscles sustain the highest flux rates.

If the high $\dot{V}_{O_2max}/M_b$ during flight in hummingbirds and insects can be fully explained by the high mitochondrial volume densities and cristae surface densities found in their flight muscles, rates of O_2 consumption per unit cristae surface area should be similar to those estimated in mammalian skeletal muscles during exercise at $\dot{V}_{O_2max}$. However, contrary to this expectation, hummingbirds and insects display higher rates of O_2 consumption per unit cristae surface area than mammals (Suarez, 1992). This is consistent with the suggestion of higher capacities for O_2 delivery. Also supporting this suggestion is the observation that cytochrome *c* oxidase, the terminal (O_2-consuming) enzyme in mitochondrial electron transport, operates closer to V_{max} in the muscles of flying honeybees than in the skeletal muscles of mammals exercising at $\dot{V}_{O_2max}$ (Suarez *et al.* 1996). In more recent work, we have determined that cytochrome turnover rates (i.e. electron flux rate per cytochrome molecule) in flying honeybees are higher than in mammalian skeletal and cardiac muscles during maximal aerobic exercise (R. K. Suarez, J. F. Staples and J. R. B. Lighton, unpublished observations).

Thus, a pattern common to glycolytic and mitochondrial enzymes has started to emerge from our comparisons of capacities with maximum loads (i.e. flux rates) at the biochemical level.

Limits and constraints

The distribution of the control of O_2 and carbon fluxes among multiple steps at all levels of biological organization and the evolutionary enhancement of flux capacities at most, if not all, of these steps in highly aerobic animals necessarily leads to the expectation that there should be many factors that might constrain the up-regulation of capacities and ultimately set upper limits to design and performance.

It has been proposed that the upper limits to the design of

O_2 transport systems may set the ultimate upper limits to maximal $\dot{V}_{O_2}/M_b$ among vertebrate homeotherms (Schmidt-Nielsen, 1984). It is possible that mass-specific cardiac outputs cannot increase further, given the already large heart mass relative to body mass and heart rates measured in shrews and hummingbirds. Further increases in hematocrit may be constrained by consequent increases in blood viscosity.

Because biological structures occupy space, the need to accommodate components that are larger or more numerous within a given volume might also set limits to the extent to which functional up-regulation is possible. Capillaries occupy approximately 10 % of flight muscle volume in hummingbirds (Mathieu-Costello *et al.* 1992; Suarez *et al.* 1991). Hummingbird flight muscles possess 25 km of capillary length and 3500 cm^2 of capillary surface area per milliliter of mitochondrial volume compared with 10 km ml^{-1} and 1400 $cm^2\,ml^{-1}$, respectively, in mammals (Hoppeler *et al.* 1997). Among vertebrates and insects possessing synchronous flight muscles, volume densities of sarcoplasmic reticulum increase with average contraction frequencies (Josephson and Young, 1987; Rome *et al.* 1996). At the higher contraction frequencies that characterize the flight of hummingbirds and such insects, a greater fraction of the ATP synthesized by mitochondria would be used by the sarcoplasmic reticulum Ca^{2+}-ATPase. This may contribute towards the low energetic efficiencies of such muscles and set a limit to how far evolution can go in the direction of higher frequencies and metabolic rates. Mitochondrial volume densities in hummingbird flight muscles are amongst the highest known for vertebrate locomotory muscles (Suarez, 1992; Suarez *et al.* 1991). Higher mitochondrial volume densities would result in less space for myofibrils and the other cytosolic components required for contractile function. Thus, higher mitochondrial volume densities are only found in modified muscles that do little or no mechanical work (e.g. brain and eye heater organs in billfish and tunas) (Block, 1991). Similarly, mitochondria occupy approximately 45 % of cell volume in shrew cardiac muscles (Weibel, 1985). This is not much higher than those in hummingbird flight muscles and similar to the volume densities found in the flight muscles of bees (Casey *et al.* 1992) and flies (Suarez, 1992). Within the mitochondria, cristae surface densities in hummingbird and insect flight muscles are close to the upper limit imposed by the need for matrix space to accommodate Krebs cycle enzymes (Srere, 1985; Suarez, 1992). It has been estimated that the protein components involved in electron transport and oxidative phosphorylation occupy more than 40 % of cristae surface area in liver mitochondria (Schwerzmann *et al.* 1986). Because respiratory chain enzymes interact like ships randomly colliding in a sea of phospholipid, higher concentrations make possible higher collision frequencies and higher capacities for electron transport (Schneider *et al.* 1980). Such higher enzyme packing densities and higher flux capacities are found in muscle mitochondria (Schwerzmann *et al.* 1989). However, membrane protein packing densities probably cannot be increased without limit (Fig. 4) without adverse consequences, e.g. molecular traffic jams and excessively high energetic cost of protein turnover.

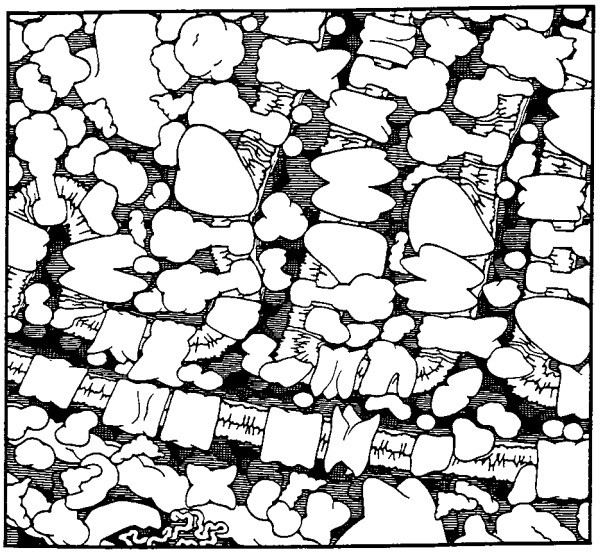

Fig. 4. A cartoon showing the tight packing of membranes and proteins within mitochondria. The outer mitochondrial membrane runs across the bottom, while the folded inner membrane is shown above. These folds form the cristae, which are shown containing high densities of enzymes and transporters involved in electron transport and oxidative phosphorylation. Reproduced from Goodsell (1993), with permission from Springer-Verlag.

At a given rate of dietary intake, increased allocation of material (e.g. proteins and fats) towards hypertrophy of certain organs and the enhancement of a specific function will result in less material being available for other organs and functions. Rates of feeding and digestion can, of course, be increased. However, even dietary intake rates cannot be increased without limit (Diamond and Hammond, 1992; Hammond and Diamond, 1997). The extent to which material costs might constrain the up-regulation of $\dot{V}_{O_2}/M_b$ has not been considered. It has been argued that large organs are energetically expensive to build and maintain. Among birds, active individuals displaying higher 'field metabolic rates' possess larger metabolically active organs and display higher basal metabolic rates than individuals with lower rates of metabolism (Daan *et al.* 1990). Flightless rails on islands without predators are thought to have lost the ability to fly in their evolution from mainland ancestors (Trewick, 1997). Smaller, less aerobic flight muscles are presumed to result in energetic savings. In juvenile 'grower' chickens, artificial selection for higher rates of muscle growth has resulted in greater pectoral muscle mass and more rapid synthesis of the enzyme glycogen phosphorylase (Flannery *et al.* 1992). Protein synthesis costs 5 ATP equivalents per peptide bond (Hawkins, 1991). On this basis, it can be calculated that both larger muscle mass and higher rates of synthesis contribute towards a 10-fold greater cost of glycogen phosphorylase synthesis than in 'layer' chickens of the same age (Suarez, 1996).

Conclusion

Animals are so well integrated in their design that, by studying them within the confines of traditional disciplines (e.g. biochemistry, organismal physiology, cell biology), we hardly do better than the six blind men groping at various parts of the elephant. Fig. 1, a schematic diagram of a hummingbird modified from Weibel's cartoon of a frog (Weibel, 1985), shows the pathway of O_2 from the external environment, through the lungs and cardiovascular system and into the muscle mitochondria. The cycle frequency of the flight muscles determines the rate of ATP hydrolysis which, in turn, determines the rate of mitochondrial ATP synthesis. These determine the steady-state rate of O_2 flux through the system. In the same way that a clock will not work if its parts do not fit properly, integrated animal function (e.g. the hovering flight of the hummingbird) would be precluded by improperly matched functional capacities. Even the hummingbird small intestine is up-regulated in its capacity for glucose transport (Diamond *et al.* 1986) to meet the great demands of its fuel-hungry metabolic machinery.

There are, no doubt, many different factors that contribute to establishing the ultimate upper limits to $\dot{V}_{O_2}/M_b$ among vertebrates and insects. Similarly, multiple factors probably contribute to set the lower limit to adult body mass among vertebrate homeotherms. Because $\dot{V}_{O_2}/M_b$ values at rest and during maximal exercise, along with associated structures and functional capacities, increase with decreasing body mass, the factors that set the upper limits to $\dot{V}_{O_2}/M_b$ must also be involved in setting the lower limit to vertebrate homeotherm body mass. It is significant that the smallest vertebrate homeotherms, including Thai bumblebee bats, Etruscan shrews and Cuban bee hummingbirds, all weigh approximately 2 g as adults. One would imagine that smaller birds and mammals would have evolved, if this were possible. Sphingid moths display metabolic rates similar to those of hummingbirds of the same body mass (Bartholomew and Casey, 1978). However, species of sphingid moths exist which weigh much less than 2 g and achieve higher body-mass-specific rates of metabolism than hummingbirds. Many species of flying insects, possessing either synchronous or asynchronous muscles, exceed the apparent limits to vertebrate homeotherm size and metabolic rate during flight. It is thought that these metabolic rates, probably the highest in the Animal Kingdom, are made possible *via* a higher-capacity tracheal system for O_2 transport (Weis-Fogh, 1964) along with enhanced capacities for fuel catabolism (Sacktor, 1976).

The arguments presented by various authors and summarized here are mainly qualitative. Clearly, we have barely scratched the surface, and much remains to be done to clarify the relationships between capacities and loads in animals. Detailed studies to explore design limits and constraints in nature's most metabolically active animals have barely begun. It is likely that control analyses at the organismal (e.g. Wagner, 1996) and biochemical (e.g. Kashiwaya *et al.* 1994) levels, as well as continued efforts towards integration, will play important roles and lead to further understanding in the years to come.

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