Biochem. J. (1992) 284, 1-13 (Printed in Great Britain)

REVIEW ARTICLE

Control of respiration and ATP synthesis in mammalian mitochondria and cells

Guy C. BROWN
Department of Biochemistry and Molecular Biology, University College London, Gower Street, London WC1E 6BT, U.K

INTRODUCTION

This article reviews recent advances in the understanding of the short-term control of mitochondrial respiration and ATP synthesis in isolated mitochondria, cells and tissues of mammals. Control of non-mitochondrial ATP synthesis, and control involving changes in gene expression and protein levels, are not covered. Other recent reviews of the topic of this article are McCormack *et al.* (1990) focusing mostly on calcium control, Brown *et al.* (1990*b*) for control by the proton gradient, Westerhoff (1989) for application of metabolic control theory and irreversible thermodynamics, Brand & Murphy (1987) focusing mostly on liver, Heineman & Balaban (1990) for heart, Soltoff (1986) for kidney, Paul (1989) and Paul *et al.* (1989) for muscle.

Mitochondrial respiration and ATP synthesis are two pathways lying at the heart of metabolism. Respiration consists of the oxidation of mitochondrial NADH by oxygen, which is coupled by the electron transport chain to the pumping of protons out across the mitochondrial inner membrane, generating an electrochemical gradient of protons (Δp) consisting of a membrane potential $\Delta\psi$ and a pH gradient (ΔpH). These protons return down their gradient either via a proton leak or via the ATP synthase. The ATP synthase couples the transport of the protons across the membrane to the synthesis of ATP inside the mitochondrial matrix. The phosphate for the phosphorylation is imported into the mitochondria by the phosphate carrier, and the ATP is exported to the cytosol in exchange for ADP by the adenine nucleotide carrier (ANC). The main substrate for the respiratory chain (NADH) is supplied by glucose, fatty acids and amino acids via three interconnected pathways. The product of ATP synthesis (ATP) is used by a large number of cellular pathways; three of the major users in mammals are the actinomyosin ATPase, the Na^+/K^+-ATPase and protein synthesis. Thus cellular energy metabolism consists of a number of pathways linked by intermediates (Fig. 1), the main function of which is to liberate and distribute free energy.

The means by which mitochondrial respiration has been thought to be controlled has undergone several revisions over the last few decades. (1) Lardy & Wellman (1952) and Chance & Williams (1955, 1956) based on work with isolated mitochondria advanced the concept that mitochondrial respiration and ATP synthesis are controlled by the ATP demand of cellular ATP-utilizing reactions. The mechanism by which increased ATP use led to increased respiration was a simple substrate limitation effect: oxidative phosphorylation was limited by ADP (and P_i) concentrations and increased ATP utilization led to an increase in ADP (and P_i) levels. (2) Klingenberg (1961) and Erecinska & Wilson (1982) elaborated the 'near-equilibrium hypothesis' on the basis of thermodynamic measurements in mitochondria and cells. They argued that the whole of oxidative phosphorylation from mitochondrial NADH to cytosolic ATP operated close to equilibrium, apart from cytochrome oxidase which was far from equilibrium. Consequently mitochondrial respiration and ATP synthesis were controlled by: (*a*) the mitochondrial NADH/NAD ratio, (*b*) the phosphorylation potential, and (*c*) any effectors of cytochrome oxidase, for example pH or oxygen concentration. (3) Atkinson (1968, 1977), on the basis of the allosteric properties of isolated enzymes, suggested that energy metabolism was controlled by the adenylate energy charge {that is the parameter ([ATP]+0.5[ADP])/([ATP]+[ADP]+[AMP])}. The ATP, ADP and AMP acted as allosteric effecters of glycolytic enzymes, matrix dehydrogenases and ATP utilizing pathways. (4) Denton & McCormack (1980) and Hansford (1980), on the basis of the properties of matrix dehydrogenases when isolated and inside mitochondria, proposed that in addition to other controls, oxidative phosphorylation is controlled by cell calcium levels acting as an activator of several key matrix dehydrogenases. (5) A number of groups have suggested that mitochondrial respiration and ATP synthesis are not only regulated via the linked pathways of energy metabolism but also the components of the respiratory chain and ATP synthesis are directly regulated. Vignais (1976) suggested regulation of the adenine nucleotide carrier by fatty acyl-CoA. Kadenback (1986) suggested that cytochrome oxidase was a site of allosteric regulation. Halestrap (1989) suggested an effect of calcium on mitochondrial volume which then regulated the respiratory chain. The ATP synthase has been proposed to be regulated by inhibitory proteins, one of which is calcium-sensitive (Yamada & Huzel, 1988; Chernyak & Koslov, 1986; Das & Harris, 1990). (6) Application of the concepts and methods of metabolic control theory to isolated mitochondria by Groen *et al.* (1982) and Tager *et al.* (1983) has led to the idea that the control over mitochondrial respiration and ATP synthesis is shared by a number of reactions and pathways, and that the distribution of control changes under different metabolic conditions.

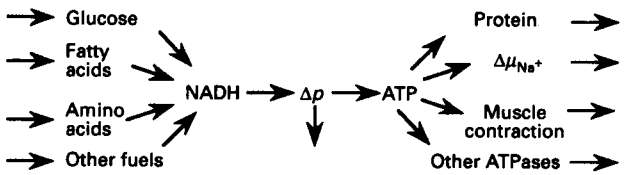

Fig. 1. The major pathways of energy metabolism and free energy dissipation

The major pathways by which free energy is funnelled and distributed through cellular metabolism are schematically illustrated. The respiratory substrates are supplied by intra- and extra-cellular pathways, and oxidized by mitochondrial NAD^+ via the interrelated pathways of glycolysis, β-oxidation, transamination and the Krebs cycle. Mitochondrial NADH is oxidized by the respiratory chain and coupled to the production of the mitochondrial proton gradient, Δp. Δp is dissipated by a mitochondrial proton leak and by ATP synthesis. ATP is used by three main pathways: muscle contraction, the Na^+/K^+-ATPase and protein synthesis, but is also used by many other cellular ATPases.

Abbreviations used: ANC, adenine nucleotide carrier; ΔG_p, phosphorylation potential; Δp, protonmotive force.

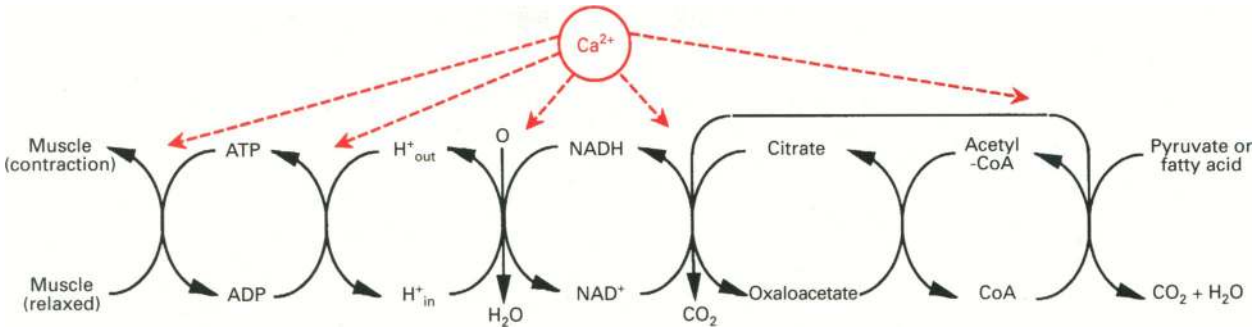

Fig. 2. Control of energy metabolism by Ca^{2+} and ADP

Energy metabolism consists of a series of moiety-conserved cycles, some of which are illustrated. An increased ADP/ATP ratio due to increased cellular ATP utilization (due to muscle contraction in this Figure) causes a decreased Δp and an increased ratio of mitochondrial NAD^+/NADH, oxaloacetate/citrate and CoA/acetyl-CoA. Calcium can stimulate muscle contraction and matrix dehydrogenases, and possibly indirectly the ATP synthase and respiratory chain.

Thus, there have been a number of suggested concepts and mechanisms of control, some compatible and complementary, some incompatible and contradictory. In order to discuss these ideas further we need to clarify certain ambiguities in the concepts and terminology of metabolic control. (1) 'Control' should be distinguished from 'regulation'. 'X controls respiration' in this article only means that a change in the level of X will cause a change in the rate of respiration, and does not necessarily imply that respiration is regulated by X *in vivo*. (2) In an experiment the metabolites directly manipulated by the experimenter are regarded as 'controlling' the dependent metabolites or fluxes which are 'controlled'. *In vivo* (i.e. in an open system) there is no master reaction or metabolite that controls respiration, since every change is caused by a change in something else. (3) Metabolite levels control enzyme activities, and enzyme activities control metabolite levels. Confusion can be avoided by talking about control of respiration by either enzyme levels or independent metabolite levels, but not both at the same time. (4) The means by which respiration is regulated may differ from tissue to tissue. (5) Because energy metabolism consists of branching pathways the control of one pathway is linked to, but not equivalent to, that of other pathways. Thus, respiration could potentially be controlled (or not controlled) by any of the pathways depicted in Fig. 1. (6) Energy metabolism does not consist of unidirectional pathways (as depicted in Fig. 1) but rather consists of a chain (or chains) or moiety-conserved cycles (some of which are depicted in Fig. 2), and this has several implications: (*a*) either end of the chain can be considered the beginning; (*b*) the rate of energy metabolism can be controlled at either end of the pathway (or in the middle) as kinetic perturbations travel in both directions more easily; (*c*) changes in the level of the conserved moieties (e.g. NAD^++NADH) could control the rate of energy metabolism.

The rest of this article discusses: (i) how mitochondrial respiration and ATP synthesis are controlled by their substrates and products, (ii) which enzymes limit (i.e. control) the rates, and (iii) how respiration and ATP synthesis are regulated by ions and metabolites acting as effectors on the pathways of energy metabolism.

CONTROL BY SUBSTRATES AND PRODUCTS

Mechanism by which ATP utilization controls ATP supply

Following the work of Chance & Williams (1955, 1956) it is well-established both in isolated mitochondria and in cells that increased ATP usage can increase mitochondrial ATP synthesis, respiration and substrate utilization, simply by providing the substrates (ADP and P_i) for oxidative phosphorylation, and mass action stimulates the chain all the way back to respiratory substrate oxidation.

Increased ATP usage in the cytoplasm decreases extramitochondrial ATP levels and increases ADP and P_i levels (see Fig. 7). The percentage changes in free ADP and P_i seen *in vivo* are usually larger than the percentage changes in ATP, but the absolute changes is ADP level are smaller because over 90% of the ADP in bound (e.g. Brindle *et al.*, 1989). The flux through the adenine nucleotide carrier is sensitive to the mitochondrial and cytosolic free ADP and ATP levels, and to the mitochondrial membrane potential as the carrier is electrogenic (Klingenberg, 1985). Increased cytosolic ADP stimulates ADP uptake in exchange for ATP export, increasing the matrix ADP/ATP ratio. The rate of the mitochondrial ATP synthase is sensitive to the intramitochondrial levels of ADP, ATP, P_i, and to Δp (van Dam *et al.*, 1981; Kagawa, 1984; La Noue *et al.*, 1986). Phosphate is imported electroneutrally with a proton. Phosphate carrier activity greatly exceeds the net rate of ATP synthesis at least in liver, and the carrier is thought to operate close to equilibrium in mammals (Ligeti *et al.*, 1985).

Increased ATP synthesis plus ADP and P_i transport results in proton influx and a decrease in Δp, measured in isolated liver mitochondria (Nicholls, 1974) and cells (see Fig. 6; Brown *et al.*, 1990*c*). A decreased Δp elicited by ATP turnover or more directly by proton ionophores has been shown to stimulate the respiratory chain in isolated liver mitochondria (Nicholls, 1974) and cells (see Fig. 6; Brown *et al.*, 1990*c*). Decreased $\Delta\psi$ or ΔpH stimulates cytochrome oxidase (Moroney *et al.*, 1984; Brown & Brand, 1985; Murphy & Brand, 1987; Capitanio *et al.*, 1990), the cytochrome bc_1 complex (Papa *et al.*, 1981; Brown & Brand, 1985) and probably the NADH–ubiqinone oxidoreductase (complex I) (Rottenberg & Gutman, 1977; Brown & Brand, 1988). Cytochrome oxidase and the bc_1 complex are also sensitive to the cytochrome *c* redox state (Brown & Brand, 1985; Murphy & Brand, 1987; cytochrome oxidase control was reviewed by Cooper, 1990). NADH–ubiqinone oxidoreductase and the bc_1 complex are sensitive to the ubiquinone redox state (Klingenberg, 1968; Rottenberg & Gutman, 1977; Brown & Brand, 1985, 1988). Increased activity of the respiratory chain results in oxidation of the mitochondrial NADH as can be seen in isolated mitochondria on addition of ADP or a protonophore (see Klingenberg, 1968; Hansford, 1980) and in liver cells (see Fig. 3). A decreased mitochondrial NADH/NAD^+ ratio increases the oxaloacetate/citrate ratio by stimulation of the three NAD-linked dehydrogenases of the Krebs cycle, resulting in a stimu-

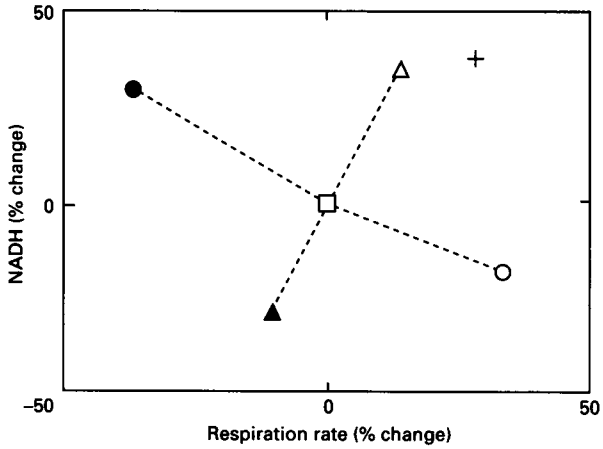

Fig. 3. Relation between the respiration rate of liver cells and the level of mitochondrial NAD(P)H measured by fluorescence

Isolated liver cells respiring on glucose in the absence of other effectors (□), were titrated with β-hydroxybutyrate (△) to increase mitochondrial NADH supply, acetoacetate (▲) to decrease mitochondrial NADH supply, gramicidin (○) to increase NADH oxidation or myxothiazol (●) to decrease NADH oxidation. Gramicidin is a Na^+ ionophore and increases respiration by stimulating ATP use by the Na^+/K^+-ATPase. Myxothiazol is an inhibitor of the respiratory chain. The relative control coefficients of NADH supply and NADH oxidation over respiration are given by the relative gradients of the lines, and were roughly 0.25:0.75. Addition of a calcium-mobilizing hormone vasopressin (+) stimulates respiration mainly by increasing NADH supply. Adapted from Brown *et al.* (1990*c*).

lation of the Krebs cycle (see Hansford, 1980). Increased Krebs cycle activity lowers the acetyl-CoA/CoA ratio (seen in isolated mitochondria on transition from State 4 to State 3; Hansford & Johnson, 1975). A decreased mitochondrial acetyl-CoA/CoA ratio and/or NADH/NAD ratio stimulates both pyruvate dehydrogenase (see Hansford, 1980) and β-oxidation of fatty acids (Latipaa *et al.*, 1986).

There has been some controversy as to whether changes in the rate of ATP synthesis due to increased ATP utilization are normally caused by the changes in the concentrations of ATP, ADP or P_i or some combination of these metabolite changes. With isolated mitochondria of liver or heart, incubated under a range of conditions, respiration has been found to correlate best with either [ADP] (Jacobus *et al.*, 1982), the [ADP]/[ATP] ratio (Davis & Lumeng, 1975; Davis & Davis–Van Theinen, 1978; see Tager *et al.*, 1983 for review) or $[ADP]\cdot[P_i]/[ATP]$ (or the logarithm of this parameter, i.e. the phosphorylation potential ΔG_p) (Forman & Wilson, 1983; Gyulai *et al.*, 1985; see Erecinska & Wilson, 1982 for review). If oxidative phosphorylation were close to equilibrium (apart from cytochrome oxidase) as found in mitochondria and cells in some conditions (see Klingenberg, 1961; Wilson & Erecinska, 1982) then we might expect the rate of respiration and ATP synthesis of isolated mitochondria to be limited by cytochrome oxidase, and the respiration rate to correlate with ΔG_p. However, proximity to equilibrium does not guarantee that a step will not have some control over pathway rates (see Kacser & Burns, 1973). If on the other hand the ANC operates far from equilibrium and limits the rates of respiration and ATP synthesis, as found with isolated mitochondria in some conditions (see Letko *et al.*, 1980; Tager *et al.*, 1983), then we might expect the rates to better correlate with [ADP] or the [ADP]/[ATP] ratio. In isolated mitochondria it has been found that both cytochrome oxidase and the ANC (and other reactions) may partially (but not fully) limit respiration, and the degree of limitation depends on the conditions and the tissue type (see below). Thus, it is not unexpected that the degree to which respiration correlates with [ATP], [ADP] and $[P_i]$ also varies. However, what evidence there is from cells and tissues (see Erecinska & Wilson, 1982) indicates that oxidative phosphorylation is close to equilibrium (apart from cytochrome oxidase).

Changes in P_i, ADP and ATP concentration around their physiological levels can each change respiration rate. However, physiological percentage changes in ATP with increased ATP utilization are usually small compared to percentage changes in phosphate and free ADP, particularly in tissues where the [ATP] is buffered by creatine phosphate (e.g. Brindle *et al.*, 1989) and the ATP sensitivity of adenine nucleotide carrier may not be as high as previously thought (Jacobus *et al.*, 1982). The phosphate sensitivity of respiration is significant but at physiological concentrations is not usually as great as the ADP sensitivity, at least in isolated mitochondria (Kunz *et al.*, 1981; Forman & Wilson, 1983; Chance *et al.*, 1985). Thus the response of respiration to changes in ATP utilization may be dominantly caused by changes in [ADP]; however, the response is likely to vary with tissue and conditions. Brindle *et al.* (1989) set variable steady state work rates of rat hind limb *in vivo* and found ATP synthesis was proportional to free ADP concentration (and P_i) measured by ^{31}P-n.m.r., up to but not including the maximal work rate, while ATP changed little (Fig. 7). On the other hand Funk *et al.* (1990) found that the work/rest transition of skeletal muscle was best modelled assuming respiration was linear with the phosphorylation potential (or some equivalent parameter), rather than with ADP concentration.

Control by respiratory substrate supply

Increasing the supply of respiratory substrate can stimulate respiration in isolated mitochondria and some tissues. The mechanism of this effect is again a mass action effect along the chain of moiety-conserved cycles (Fig. 2). Increase in mitochondrial NADH supply increases the mitochondrial $NADH/NAD^+$ ratio which stimulates respiration, raising Δp and thus stimulating the proton leak and phosphorylation, resulting in an increased ΔG_p which may (or may not) then stimulate ATP-utilizing reactions (depending on their sensitivity to ΔG_p). Changes in respiratory substrate concentration and type (addition of pyruvate, fatty acids or β-hydroxybutyrate) have been found to change the mitochondrial $NADH/NAD^+$ ratio and respiration rate in phosphorylating isolated rat liver mitochondria (Koretsky & Balaban, 1987), in isolated rat heart mitochondria (Moreno-Sanchez *et al.*, 1990), in isolated rat liver cells (see Fig. 3; Brown *et al.*, 1990*c*; Nobes *et al.*, 1990*b*), in rat brain synaptosomes (Kauppinen & Nicholls, 1986*a*), and in rabbit proximal tubules (Balaban & Mandel, 1988). However, Kim *et al.* (1991) found that although increased NADH supply increased the ATP/ADP ratio in rat heart *in vivo* it did not increase oxygen consumption.

In all the above cases the changes in substrate concentration are large compared to the changes in respiration rate, implying that, although substrate supply can control respiration in isolated mitochondria and some tissues, the control is small relative to that by ATP utilization (as shown in isolated liver cells by Brown *et al.*, 1990*c*). Changes in glucose concentration around the physiological level do not normally significantly change tissue respiration rates (e.g. Siesjo, 1978), implying that glycolysis is normally saturated with substrate. Increases in fatty acid concentration may stimulate respiration in mitochondria and tissues by increasing NADH supply, but they also increase respiration by other effects (see below). Fatty acids and glucose compete as respiratory substrates, and suppress each other's oxidation more

dramatically than they change respiration (see Sugden *et al.*, 1989).

Fatty acid oxidation by mitochondria is thought to be limited by (*a*) fatty acid supply, (*b*) ATP utilization and (*c*) (malonyl-CoA-inhibited) carnitine palmitoyltransferase (CPT1) (reviewed in McGarry & Foster, 1980; Bremer, 1983; Bremer & Osmundsen, 1984). In liver malonyl-CoA regulates fatty acid oxidation and is synthesized by acetyl-CoA carboxylase, and this enzyme (and therefore fatty acid synthesis and oxidation) is regulated by the level of fatty acids, citrate, glucagon and insulin. In many other tissues CPT1 is also malonyl-CoA sensitive, but the role of such inhibition is less clear (Saggerson, 1986).

Control by oxygen

The apparent K_m of isolated cytochrome oxidase for oxygen in the absence of Δp is about 1 μM (Petersen *et al.*, 1976) and the apparent K_m of respiration in isolated mitochondria for oxygen has been measured to be between 0.01 and 1 μM, being higher in coupled than uncoupled mitochondria (Oshino *et al.*, 1974; Petersen *et al.*, 1974; Wilson *et al.*, 1979, 1988). The oxygen concentration of venous blood is considerably higher than these values; thus it might be thought that cytochrome oxidase is always saturated with oxygen. However, Wilson *et al.* (1988) found that although the oxygen concentration required for half maximal respiration in isolated mitochondria is close to 1 μM in State 4, the ratio of reduced/oxidized cytochrome *c* begins to rise below 20 μM-oxygen. This may be because cytochrome oxidase has little control over State-4 respiration rate at high oxygen concentrations, so that although cytochrome oxidase is inhibited at 5–20 μM-oxygen there is little change in respiration until oxygen is below 5 μM. Tissue oxygen levels have been measured in perfused organs and intact animals with micro-oxygen electrodes, and have been found to be very heterogeneous in the same tissue. Typical values are < 1 to 90 μM with a median of about 35 μM in rat liver, kidney and brain (see Kessler *et al.*, 1973; Vanderkooi *et al.*, 1991). These values are thought to reflect cytosolic levels. Whether significant oxygen gradients exist between the cytosol and the mitochondria has been the subject of conflicting reports, but recent evidence suggests these gradients are small (reviewed in Jones, 1986; Wittenberg & Wittenberg, 1989; Tamura *et al.*, 1989). Thus, there are steep oxygen gradients in tissues resulting in considerable heterogeneity in local oxygen concentration, and these gradients change with the energy turnover of the tissue. Most cells have oxygen concentrations well above those limiting respiration, but in some organs and conditions a small and variable proportion of the cells or mitochondria may have a local oxygen concentration such that redox state of cytochrome oxidase or the respiration rate itself is partially oxygen limited.

Different organs show different responses to changes in oxygen supply. In heart, increasing oxygen supply above the physiological level results in a small increase in oxygen consumption but the mechanism of this stimulation is unclear (Oguro *et al.*, 1973). In brain, oxygen consumption is independent of oxygen supply down to very low levels (Kinter *et al.*, 1984; Siesjo, 1978). In liver and kidney, reduction in oxygen supply below the physiological levels initially has no effect on oxygen consumption, but consumption begins to decline even at fairly high levels (Edelstone *et al.*, 1984; Bauer & Kurtz, 1989). Tissues that show no change in oxygen consumption with changes in oxygen delivery may still show adaptive changes in metabolite levels (see Jones, 1986; Tamura *et al.*, 1989). For example, small increases in oxygen delivery to human brain *in vivo* result in oxidation of brain cytochrome oxidase measured by near-infrared spectroscopy (Hampson *et al.*, 1990; Edwards *et al.*, 1991). The maximal oxygen consumption of skeletal muscle is proportional to oxygen supply without attainable saturation (Whalen *et al.*, 1973), and maximal potential oxygen use by skeletal muscle far exceeds the capacity of the heart to supply it; thus maximal muscle activity and body oxygen consumption is limited by cardiac output and oxygen supply to the muscle (see Astrand, 1989).

APPLICATION OF METABOLIC CONTROL THEORY

Which reactions limit the rate of respiration and ATP synthesis?

Changes in the effective level (by changing enzyme concentration or effector level) of which enzymes will change respiration rate? The degree of rate limitation can be quantified as the flux control coefficient of the enzyme, in the terminology of metabolic control theory (see Kacser & Burns, 1973; Westerhoff, 1989; Cornish-Bowden & Cardenas, 1990). The flux control coefficient is the percentage change in the steady state rate of the pathway divided by the percentage change in the enzyme level causing the flux change. The coefficient is defined for infinitesimally small changes, and therefore only refers to one particular state of the pathway; in other states the coefficients may be different. The sum of the control coefficients of all the enzymes in a system always adds up to 1.

In a pioneering application of metabolic control theory Groen *et al.* (1982) measured the control coefficients of mitochondrial enzymes/transporters over respiration in isolated liver mitochondria, using specific inhibitors to alter the effective level of the enzymes. They found that control was shared, and the distribution of control changed depending on the amount of ATP-utilizing enzyme (hexokinase) added to the mitochondria and thus the rate of ATP turnover. The ANC, respiratory substrate transport and cytochrome oxidase had most of the control in State 3 (maximum rate of ATP turnover), while at intermediate rates of ATP turnover the ANC, respiratory substrate transport and the added ATP-utilizing enzyme (hexokinase) had most of the control (see Fig. 5). Groen *et al.* (1982) indicated that the proton leak has all the control in State 4 (when there was no ATP utilization). However, Brand *et al.* (1988) later showed that the derived control coefficient of the proton leak was an artifact of the assumptions made, and that the respiratory chain has some control over respiration rate even in State 4. A number of other estimates of control coefficients over State-3 respiration in isolated mitochondria have been made (Forman & Wilson, 1983; Davies & Davies–Van Thienen, 1984; Doussiere *et al.*, 1984; Verhoeven *et al.*, 1985; Moreno-Sanchez, 1985; Kunz *et al.*, 1988; Moreno-Sanchez *et al.*, 1988, 1991; Willis & Dallman, 1989). In liver all the component enzymes and transporters of oxidative phosphorylation (except the phosphate carrier) have been reported to partially limit respiration in some conditions, but the extent to which different steps limit the rate varies dramatically with different conditions. In isolated heart mitochondria, complex 1 and the ATP synthase may have most of the control over State-3 respiration (Doussiere *et al.*, 1984; Moreno-Sanchez *et al.*, 1991). Many of these studies suffered from the use of unphysiological conditions: low temperatures, saturating concentrations of unphysiological substrates, low external adenine nucleotide concentrations, and unrepresentative ATP-using reactions. The distribution of control has been found to depend on the concentrations of externally added adenine nucleotides and phosphate (Doussiere *et al.*, 1984; Kunz *et al.*, 1988). Moreno-Sanchez *et al.* (1991) found that the control distribution in heart and kidney mitochondria depended on substrate and calcium concentrations. Doussiere *et al.* (1984) showed that the distribution of control over intermediate rates depended on the kinetics of the added ATP-using enzyme, i.e. the relative control by ATP supply and ATP usage depended on the relative

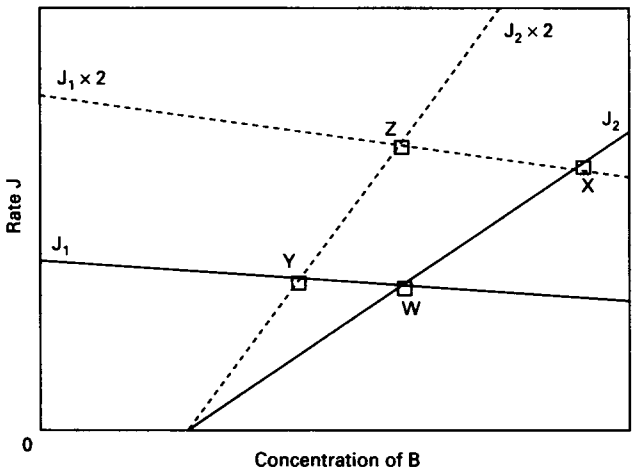

Fig. 4. Illustration of the effect of doubling enzyme concentrations on the steady state flux and intermediate concentration of a hypothetical two-enzyme pathway

The solid lines (J_1 and J_2) give the hypothetical relations between the concentration of intermediate metabolite B and the rates of enzymes 1 and 2. The intercept of these lines (W) gives the steady state for a pathway of these two enzymes. The broken lines give the concentration-rate relations for double the original amount of enzymes 1 or 2, with new steady states at X, Y and Z. The relative control coefficients of enzymes 1 and 2 over the pathway flux is given by the inverse of their relative sensitivities to metabolite B (i.e. the gradients of the lines at W). From the graph the flux control coefficients would be 0.9 for enzyme 1 and 0.1 for enzyme 2.

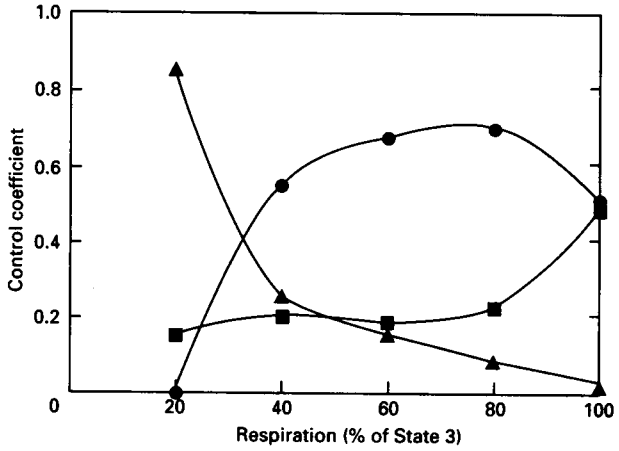

Fig. 5. Control coefficients of various pathways over the respiration rate of isolated mitochondria at different rates between State 4 and 3

The control coefficients are of the respiratory chain (and substrate transport) (■), the proton leak (▲) and phosphorylation (ATP synthesis, transport and hydrolysis by hexokinase) (●). Adapted from Hafner *et al.* (1990*b*).

sensitivities of these two processes to the ATP/ADP ratio. Thus the control distribution in cells would depend on the sensitivity of the ATP using reaction to ATP/ADP.

The relative sensitivities of different pathway enzymes to their substrates and products determines their relative control coefficients (Kacser & Burns, 1973). We can illustrate this with a hypothetical two-enzyme pathway (see Fig. 4):

$$A \xleftrightarrow{1} B \xleftrightarrow{2} C$$

where pathway substrate A is converted to intermediate metabolite B by enzyme 1, and enzyme 2 converts B to pathway product C. If metabolite concentrations [A] and [C] are fixed and the dependence of the rates of enzymes 1 and 2 on metabolite [B] are as depicted in Fig. 4, then the steady-state rate of the pathway is given by the intercept of the two lines, i.e. W in Fig. 4. If the level of enzyme 1 is doubled the rate of enzyme 1 doubles at any given level of B, and the new steady state is at X in Fig. 4, i.e., the rate almost doubles. But if the level of enzyme 2 is doubled the new steady state is at Y, i.e. the rate only increases by a small amount. In fact the relative control coefficients of 1 and 2 are equal to the inverse of their relative sensitivities (elasticities) to B (the relative gradients of the lines at W). Now this is also true when 1 and 2 are not single enzymes, but two ends of a pathway and B is an intermediate (Brown *et al.*, 1990*a*). Thus, if (in Fig. 4) B were the ATP/ADP ratio, J_1 the rate of ATP synthesis and J_2 the rate of ATP usage, then the processes of ATP synthesis would have most of the control while ATP usage would have little control, and the ATP/ADP ratio would have to decline dramatically to cause any significant increase in ATP turnover. Thus, by experimentally determining the relation between pathway rates and some intermediate in mitochondria or cells it is possible to determine the flux control coefficients. This method is known as the top-down approach to metabolic control theory (see Brown *et al.*, 1990*a*). Additionally such plots can be compared in the presence and absence of some metabolic perturbation (such as addition of a hormone or metabolite) in order to determine where the perturbation has affected the pathway. This method has been used to locate where in energy metabolism the changes occur when respiration is stimulated by: glucagon (Brand *et al.*, 1990), and thyroid hormone (Hafner *et al.*, 1990*a*) in isolated mitochondria, and fatty acids (Nobes *et al.*, 1990*b*) and vasopressin (Brown *et al.*, 1990*c*; see Fig. 3) in liver cells.

Brand *et al.* (1988) and Hafner *et al.* (1990*b*) used the top-down approach to metabolic control theory to estimate the control by groups of enzymes over mitochondrial respiration and ATP synthesis. They measured the dependence of the Δp-producing processes (substrate transport, oxidation and the respiratory chain) on Δp, and the dependence of the Δp-utilizing processes (proton leak, ATP synthesis, transport and utilization) on Δp, and from this deduced the control coefficients. The proton leak had most but not all the control over respiration in state 4, but this control declined rapidly towards State 3 (see Fig. 5). Control over respiration differed somewhat from control over ATP synthesis. Most of the control over the rate of ATP synthesis, transport and utilization was in these same processes, i.e. there was little control by substrate transport, oxidation, the respiratory chain and the proton leak. Korzeniewski & Froncisz (1991) derived very similar flux control coefficients from their mathematical model of oxidative phosphorylation. A control analysis of the data of Koretsky & Balaban (1987) and Moreno-Sanchez *et al.* (1990) indicates that NADH supply does have significant but low control over State-3 respiration rates in isolated liver and heart mitochondria. Denton *et al.* (1980) and Moreno-Sanchez *et al.* (1990) also showed that sub-micromolar calcium can stimulate State-3 respiration in heart mitochondria, by stimulating NADH supply, suggesting that the calcium-sensitive matrix dehydrogenases can have significant control over respiration. Brown *et al.* (1990*c*) used the top-down approach in rat liver cells, and showed that the control distribution over respiration rate and ATP synthesis was similar to that of isolated mitochondria with a respiratory rate intermediate between States 3 and 4. Mitochondrial NADH supply had 15–30% of the control over cellular respiration rate, the respiratory chain had 0–15%, the proton leak had 22%, and ATP synthesis, transport and utilization had 49% (see Fig. 3). The rate of mitochondrial ATP synthesis was mostly (84%) controlled by

ATP synthesis, transport and utilization. Determination of control coefficient of the ANC in liver cells using specific inhibitors has given values of 0.25 over respiration rate in some conditions (Duszynski *et al.*, 1982), and close to 1 over gluconeogenesis and urea synthesis (Akerboom *et al.*, 1977).

There has been some dispute as to whether the diffusion of the components of the electron transport chain within the membrane may limit the maximal rate of electron transport (Lenaz & Fato, 1986; Chazotte & Hackenbrock, 1988*a*,*b*; Hackenbrock & Gupte, 1988; Ferguson-Miller *et al.*, 1988; Lenaz *et al.*, 1988). Recent evidence suggests that changing the diffusion coefficients within the membrane can change the maximal rate of electron transport, indicating that ubiquinone (and possibly cytochrome *c*) diffusion may partially limit this rate (Chazotte & Hackenbrock, 1988*a*,*b*, 1989; Hackenbrock & Gupte, 1988). If the diffusion of electron transport components is partially rate limiting then the respiratory complexes can not be regarded as independent metabolic steps by metabolic control theory. If they are erroneously regarded as independent then the sum of all control coefficients should be greater than 1. This danger may be greater in a sucrose medium which apparently limits diffusion and inhibits electron transport (Chazotte & Hackenbrock, 1988*a*,*b*).

Can ATP synthesis control cellular ATP utilization?

It was previously thought that cellular respiration and ATP synthesis were almost exclusively controlled by cellular ATP utilization i.e. mitochondria passively respond to changes in ATP demand. However, we have seen that these rates may also be controlled by mitochondrial activities. The relative control by the processes of ATP production and utilization over the rates of these processes depends on both the relative rates and their sensitivities (elasticities) to the intermediates ATP, ADP and P_i. If the rate of mitochondrial ATP synthesis is less sensitive to changes in ΔG_p than the various ATP-utilizing reactions then the level of mitochondria or mitochondrial activities will have a high level of control over both the rate of ATP synthesis and the rates of the ATP-utilizing processes i.e. over cell functions. The major ATP-utilizing reactions in mammals on a whole body basis are probably the myosin ATPase, the Na^+/K^+-ATPase and protein synthesis (Kelly & McBride, 1990; Clausen *et al.*, 1991), but other ATP users may dominate in particular cell types.

Isometric force generation by muscle has been shown to be inhibited by P_i in the physiological range due to an inhibition of the myosin ATPase (Cooke & Pate, 1985; Kentish, 1986), and the contractile function and ATP use of perfused heart can be sensitive to the ΔG_p (Kammermeier *et al.*, 1982; Kupriyanov *et al.*, 1991). However, in the heart *in vivo* Kim *et al.* (1991) found that raising the ΔG_p by increasing mitochondrial NADH supply did not increase respiration or contractile activity, indicating that mitochondrial processes had no significant flux control in heart. Aerobic training results in increased density of mitochondria in skeletal muscle, but it is not clear whether mitochondrial density *per se* can limit skeletal muscle function (see Astrand, 1989).

The Na^+/K^+-ATPase is the major ATPase in brain and kidney and is a significant ATP user in other cells (Clausen *et al.*, 1991). The isolated Na^+/K^+-ATPase is relatively insensitive to ATP, but the apparent sensitivity measured in cells is higher, with activity roughly proportional to ATP concentration in rat kidney proximal tubules (Soltoff & Mandel, 1984; Tessitore *et al.*, 1986), and cultured HeLa cells (Ikehara *et al.*, 1984), and a greater sensitivity in brain synaptosomes (Dagani & Erecinska, 1987; Erecinska & Dagani, 1990). Comparison of the relative sensitivity of pump activity and respiration rate to ATP concentration found in brain synaptosomes by Erecinska & Dagani (1990) indicates that mitochondrial ATP production had significant control over Na^+/K^+-ATPase activity. Measurements *in vivo* by ^{31}P-n.m.r. indicate that tissue average [ATP] changes relatively little as the ATP turnover rate varies, particularly in tissues containing creatine and creatine kinase. This suggests that in most tissues mitochondria are likely to have little control over Na^+/K^+-ATPase activity. However, local [ATP] may change more dramatically, and in some cells [ATP] can change dramatically, for example in kidney (Beck *et al.*, 1991). The Ca^{2+}-ATPase is a major ATP-using reaction in heart and skeletal muscle (Clausen *et al.*, 1991), but while the ATPases of endoplasmic and sarcoplasmic reticulum may have apparent K_m values in the mM range, their sensitivity to the ATP/ADP ratio is small relative to ATP synthesis (Corkey *et al.*, 1988; deMeis & Sorenson, 1989). However, this sensitivity may be important in hypoxia or ischaemia, and in permeabilized insulinoma cells and gastric smooth muscle cells it has been found that changes in ATP/ADP ratio in the physiological range cause significant changes in cytosolic free calcium levels (Gilbert *et al.*, 1991).

Major ATP-utilizing processes in liver are gluconeogenesis and urea synthesis. Brown *et al.* (1990*c*) showed that mitochondrial processes have significant but small control over total ATP turnover in resting liver cells, while Akerboom *et al.* (1977) showed that the adenine nucleotide carrier had a high level of control over gluconeogenesis and urea synthesis in liver cells. Gluconeogenesis in kidney can be inhibited without changing respiration by increasing the ATP consumption of Na^+ transport, but the opposite is not true, suggesting that gluconeogenesis in kidney has a relatively high sensitivity to ΔG_p (Soltoff, 1986). Protein synthesis accounts for 18–20% of whole body ATP use in mammals (Waterlow, 1984) and is very sensitive to changes in the ATP/ADP ratio *in vitro* and in cells due to the GDP sensitivity of initiation (Walton & Gill, 1976; Mendelsohn *et al.*, 1977; Hucul *et al.*, 1985; Gronostajski *et al.*, 1985). This suggests that mitochondria may have a high level of control over protein synthesis and protein levels. Thus (as suggested by Atkinson, 1977) there may be a hierarchy of ATP/ADP sensitivities among the ATP-utilizing reactions with the anabolic and less immediately essential processes such as protein synthesis and gluconeogenesis being very sensitive to the [ATP]/[ADP] ratio, while ion transport and muscle contraction are less sensitive. This implies that mitochondrial ATP production (and [calcium]) can have control over the former processes, and will have some control over respiration rate in tissues where these processes are the dominant ATP utilizing processes.

The ATP/ADP ratio might control cell functions by a variety of other means. For example, the GTP/GDP ratio has multiple effects on anabolic processes and cell function (Pall, 1985). Heart, skeletal muscle and parts of brain and kidney contain an ATP-inhibitable K^+ channel which controls plasma membrane potential (reviewed in Ashcroft, 1988). In pancreatic B cells the channel is regulated by physiological changes in ATP/ADP, due to glucose supply limiting ATP production (see Ashcroft, 1988), and there is some evidence for such regulation in kidney (Beck *et al.*, 1991). It is unclear whether significant changes in channel activity occur during physiological changes in ATP/ADP ratio in other cell types. A decreased ATP/ADP ratio generally causes an increased AMP and adenosine level. Adenosine can leave many cell types and is a powerful vasodilator in many tissues, and depressant in the brain (Su, 1985; Fredholm & Dunwiddie, 1988) and heart (Sperelakis, 1988). Thus, in the longer-term ATP production and utilization could control cellular functions via adenosine; adenosine acting to increase respiratory substrate and oxygen supply (by vasodilation) and decrease cellular ATP utilization (by depressing excitability). The ATP/ADP ratio might also conceivably regulate cell functions by ATP dependent

phosphorylation/dephosphorylation of proteins, for example in regulation of ion channels and exchangers (Tani, 1990).

REGULATION OF SUBSTRATE SUPPLY, THE RESPIRATORY CHAIN AND ATP SYNTHESIS

Calcium control of matrix dehydrogenases

Intramitochondrial calcium stimulates three matrix dehydrogenases: pyruvate dehydrogenase, (NAD^+)-isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase. This calcium control over substrate oxidation has been reviewed extensively (Hansford, 1985; McCormack & Denton, 1990; McCormack *et al.*, 1990), and only its implications for control of respiration and ATP synthesis will be examined here.

The level of free calcium in the cytoplasm is set physiologically mainly by the level of circulating calcium-mobilizing (and antagonizing) hormones and by the level of electrical excitation. The cytoplasmic free calcium level normally determines the mitochondrial matrix free calcium level via two mitochondrial transporters: an electrogenic uniporter for uptake and an electroneutral Ca^{2+}/Na^+ exchanger for efflux (Crompton, 1985). The matrix free calcium level is of the order of 100 nM–1 μM (see Crompton, 1985; Somlyo *et al.*, 1985; Andrews *et al.*, 1987; McCormack *et al.*, 1989, 1990). Increases in free calcium level in this range decrease the K_m of (NAD^+)-isocitrate and 2-oxoglutarate dehydrogenase for isocitrate and 2-oxoglutarate respectively and stimulate pyruvate dehydrogenase phosphate phosphatase which converts pyruvate dehydrogenase into the active dephosphorylated form. Physiological stimuli such as hormones or electrical excitation which raise cell calcium are found to activate pyruvate dehydrogenase in heart, muscle, brain and liver, and thus presumably all three dehydrogenases are stimulated (see Hansford, 1985; McCormack *et al.*, 1990). Hormones may also alter the kinetics of the calcium uptake and efflux pathways (Crompton, 1985).

Does physiological stimulation of these dehydrogenases by calcium actually change respiration and ATP synthesis rates? This depends on whether these dehydrogenases have significant control over cellular respiration, but this is not generally known. However, in some cells and conditions, it is known that mitochondrial NADH supply as a whole has a significant but not high level of control over respiration (see above). In isolated heart mitochondria submicromolar calcium can elicit a significant stimulation of State-3 respiration by stimulating mitochondrial NADH supply (Denton *et al.*, 1980; Moreno-Sanchez *et al.*, 1990). In liver calcium-mobilizing hormones (vasopressin, external ATP) can elicit changes in respiration and ATP synthesis by stimulating NADH supply, presumably by stimulating matrix dehydrogenases (see Fig. 3; Brown *et al.*, 1990*c*). In rat thymocytes cellular respiration rate has been reported to be insensitive to mitochondrial calcium level (Lakin-Thomas & Brand, 1988). In isolated rat brain synaptosomes, the depolarization-induced stimulation of respiration has been found to be calcium-dependent (Patel *et al.*, 1988; Dagani *et al.*, 1989) and calcium-independent (Kauppinen & Nicholls, 1986*b*; Erecinska & Dagani, 1990). Interpretation of the calcium dependence of respiration is complicated by possible calcium stimulation of ATP usage. Inhibition of mitochondrial calcium uptake (by Ruthenium Red) in heart cells or perfused heart does partially inhibit respiration and contractile force in the unstimulated heart. However, Ruthenium Red does not block the stimulation of respiration and contraction due to electrical stimulation or positive inotropic agents, although it inhibits activation of pyruvate dehydrogenase, and leads to a greater oxidation of NADH, and a greater increase in the ADP/ATP ratio (McCormack & England, 1983; Katz *et al.*, 1988; Hansford *et al.*, 1988; Unitt *et al.*, 1989). Stimulation of mitochondrial NADH supply (with β-hydroxybutyrate) in rat heart *in vivo* raises the phosphorylation potential but does not stimulate respiration (Kim *et al.*, 1991). These results suggest that in heart matrix calcium has relatively little control over ATP turnover via the matrix dehydrogenases, but it has significant control over the ATP/ADP ratio. This has led to the concept that the function of calcium control of matrix dehydrogenases is not to control ATP turnover, but rather to prevent a deleterious fall in the ATP/ADP ratio by simultaneoulsy stimulating ATP production and utilization. A large fall in ATP/ADP ratio might inhibit muscle contraction, protein synthesis, the Na^+/K^+-ATPase and Ca^{2+}-ATPase (see above). An alternative function for calcium control of the dehydrogenases would be to prevent excessive wastage of free energy via the mitochondrial proton leak (see below). It is in general presumed that calcium will only have a regulatory role in stimulated cells (in contrast to resting cells) as it is likely that the dehydrogenases have much lower control over respiration in resting cells.

Control over the respiratory chain by mitochondrial volume and calcium

Halestrap has provided evidence that in liver the mitochondrial respiratory chain is stimulated by calcium-mobilizing hormones acting via changes in mitochondrial volume (reviewed in Halestrap, 1989; McCormack *et al.*, 1990). The proposed chain of events is as follows. Hormones or other stimuli raise cytosolic calcium, leading to a rise in mitochondrial matrix calcium. The raised [calcium] inhibits a matrix pyrophosphatase (K_i 3–4 μM; Davidson & Halestrap, 1989) leading to a rise in matrix [pyrophosphate] (shown in liver mitochondria exposed to micromolar calcium; Davidson & Halestrap, 1987). The raised matrix [pyrophosphate] is suggested to stimulate K^+ uptake into the mitochondria, leading to an increase in mitochondrial volume. Addition of calcium or pyrophosphate, or other agents that raise mitochondrial pyrophosphate, was found to increase the volume of isolated liver mitochondria (Davidson & Halestrap, 1987) and heart mitochondria (Halestrap, 1987). The increased mitochondrial volume stimulates the respiratory chain (by an undefined mechanism) at the level of electron flow into the ubiquinone pool, resulting in stimulation of respiration on many different substrates, particularly fatty acids, in liver and heart mitochondria (Davidson & Halestrap, 1987; Halestrap, 1987). However, it has been found that sucrose (which was used by Halestrap to change the volume of isolated mitochondria) can inhibit respiration of inner membrane vesicles apparently by a viscosity effect on membrane mobility rather than via a volume effect (Chazotte & Hackenbrock, 1988*a*,*b*). Also, the calcium stimulation of mitochondrial volume might result from calcium stimulation of matrix dehydrogenases, raising $\Delta\psi$ and thus stimulating the normal K^+ uniport activity known to be very sensitive to $\Delta\psi$ (Brown & Brand, 1986).

The evidence for the chain of events suggested by Halestrap (1989) in more intact systems is limited as yet. In isolated liver cells vasopressin addition has been found to cause an increase in mitochondrial pyrophosphate (Davidson & Halestrap, 1988) and volume (Quinlan & Halestrap, 1986; however, see also Brown *et al.*, 1990*c*), and the initial increase in NADH/NAD^+ ratio (thought to be due to calcium activation of matrix dehydrogenases) is followed by a partial decrease in the ratio (proposed to be due to activation of the respiratory chain) (Quinlan & Halestrap, 1986). Brand *et al.* (1990) found that the activation of respiration of liver mitochondria from rats treated with glucagon (which can act as a calcium-mobilizing hormone in liver) was due to stimulation of electron flux from succinate to ubiquinone, consistent with the suggested mechanism of Halestrap (1989).

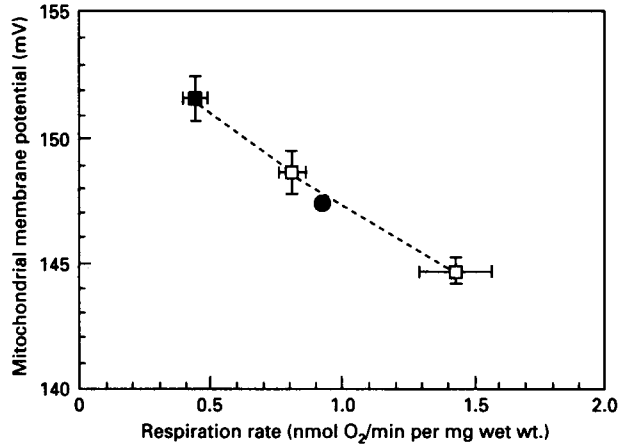

Fig. 6. Relation between the respiration rate of liver cells and the mitochondrial membrane potential

Isolated liver cells respiring on glucose in the absence of other effectors (●) were treated with excess oligomycin (■) to inhibit all mitochondrial ATP synthesis, and were then titrated with two concentrations of the proton ionophore FCCP (□) to partially uncouple the mitochondria. From Brown *et al.* (1990*c*).

Control over the ATP synthase by inhibitory proteins and calcium

The ATP synthase can have significant control over respiration and ATP synthesis in isolated liver and heart mitochondria in some conditions (see above). Measurements of the forward and reverse rate constants in isolated liver and heart mitochondria also indicate that the forward rate greatly exceeds the reverse rate in normal conditions, so that the net rate can be regulated (LaNoue *et al.*, 1986). The synthase can be inhibited (additively) by two distinct inhibitor proteins, simultaneously present in the heart and skeletal muscle of larger mammals (Chernyak & Koslov, 1986; Yamada & Huzel, 1988; Das & Harris, 1990). The inhibitor protein isolated by Pullman & Monroy (1963) appears to only bind and inhibit synthase as low Δp, and is also regulated by pH, ATP and redox state (Chernyak & Koslov, 1986; Lippe *et al.*, 1988), and may be involved in preventing ATP hydrolysis by the synthase during hypoxia. The inhibitor protein isolated by Yamada & Huzel (1988) binds calcium and is removed from the synthase by about 1 μM calcium, allowing increased rates of ATP synthesis and hydrolysis (Yamada & Huzel, 1985, 1988). Das & Harris (1989) found that the ATPase activity of isolated heart cells fell by about 40% after anoxia and was raised reversibly by 70% by electrical stimulation of the cells (with a half time of 20 s). These experiments suggest that calcium control of the ATP synthase activity may well be important in some tissues.

Control over matrix dehydrogenases by adenine nucleotides

A decreased intramitochondrial ATP/ADP ratio activates pyruvate dehydrogenase (by changing its phosphorylation state), and (NAD^+)-isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase (by changing substrate K_m) (Hansford, 1980). However, in mammals this direct control by ATP/ADP is thought to be relatively unimportant in causing the work-rest transition of dehydrogenase activity, in comparison to the effects of $NADH/NAD^+$ (and acetyl-CoA/CoA for pyruvate dehydrogenase) (see Hansford & Johnson, 1975; Hansford, 1980). Brown *et al.* (1990*c*) investigated this effect in liver cells by comparing the respiration rates of cells with different ATP/ADP ratios but manipulated to have the same mitochondrial Δp (see Fig. 6). They found no significant effect of ATP/ADP ratio on Δp production or respiration, although there may be a small effect on NADH production. The sensitivity of isocitrate and oxoglutarate dehydrogenses to calcium is increased by a decreased ATP/ADP ratio (Rutter & Denton, 1988) but the quantitative significance of this effect is unknown.

Control over cytochrome oxidase

Kadenbach (1986) suggested that cytochrome oxidase might be a site for allosteric regulation, on the basis that cytochrome oxidase operates far from equilibrium, has significant control over respiration rate in isolated mitochondria, and has multiple tissue specific subunits with no known catalytic function. However, there are no known significant physiological effectors of cytochrome oxidase. ATP, ADP and P_i may alter cytochrome oxidase kinetics, but the physiological changes do not have a significant effect (Kadenbach, 1986). Increased pH inhibits the oxidase over the physiological range (Wilson *et al.*, 1988; Cooper, 1990), but physiological changes in cytosolic pH are normally small relative to the sensitivity of the oxidase to pH. Isolated oxidase is normally present in both 'slow' and 'fast' forms which may be interconverted, but the physiological significance of these forms is doubtful as the 'slow' form is not found in isolated mitochondria (see Moody *et al.*, 1991).

Control over the adenine nucleotide carrier

The ANC can have a high degree of control over respiration (and ATP synthesis) in isolated mitochondria and cells of rat liver, but may have less control in other organs (see above). The carrier can be inhibited by fatty acyl-CoA in isolated mitochondria (see Vignais, 1976) but evidence for this action *in vivo* is limited. Soboll *et al.* (1984*b*) showed that conditions (starvation or addition of oleate) that raised the fatty acyl-CoA content of liver in intact rats, in perfused liver and isolated liver cells caused a decrease in liver cytosolic ATP/ADP ratio and an increase in mitochondrial ATP/ADP ratio. However, these condition generally increase respiration rate due to other effects of fatty acids, and this suggests that the ANC is inhibited but has no significant control over respiration. In contrast Akerboom (1977) and Duszynski *et al.* (1982) found significant control by the adenine nucleotide carrier over gluconeogenesis and respiration in liver cells but no effect of raising fatty acyl-CoA (by adding oleate) on the control coefficient of the carrier, suggesting no inhibition by fatty acyl-CoA. However, the effects of fatty acids on liver metabolism are multiple and difficult to disentangle at present (see Brand & Murphy, 1987; Schonfeld *et al.*, 1988; Nobes *et al.*, 1990*b*). Lochner *et al.* (1981) found that increased content of acyl-CoA in isolated rat hearts during ischaemia actually increased ANC activity.

The mitochondrial proton leak

The mitochondrial inner membrane has a proton leak, so that a proportion of the protons pumped by the respiratory chain return via this leak rather than driving ATP synthesis and ATP transport (reviewed in Murphy, 1989; Brand, 1990). In mitochondria (other than brown adipose tissue mitochondria) the leak may be mainly via the bilayer (Brown & Brand, 1991). The proton leak rate is greatly increased at high Δp in isolated mitochondria (Nicholls, 1974; Brown & Brand, 1986; Brown, 1989) and liver cells and thymocytes (Nobes *et al.*, 1990*a*; Brown *et al.*, 1990*c*). In isolated mitochondria the State-4 respiration rate is mainly controlled by the proton leak (Brand *et al.*, 1988), while the leak has relatively little control over the rate of ATP synthesis and State-3 respiration (Hafner *et al.*, 1990*b*). The leak accounts for 20–30% of the basal respiration rate of isolated liver cells (Nobes *et al.*, 1990*a*; Brown *et al.*, 1990*c*) and has significant control over this rate (Brown *et al.*, 1990*c*).

Free fatty acids increase the proton permeability of isolated mitochondria, whether added externally or generated internally (Borst *et al.*, 1962), and act as natural protonophores and uncouplers (Gutknecht, 1988). High levels of free fatty acids can uncouple cells and perfused tissues (Soboll *et al.*, 1984*a*), and such uncoupling may occur in some pathological states, such as ischaemia. Whether fatty acids significantly enhance the endogenous proton leak in physiological states is unclear. Brown & Brand (1991) found that about 25% of the proton leak in freshly isolated liver mitochondria from fed rats was due to fatty acids, but these fatty acids might have been generated during preparation of the mitochondria. Klug *et al.* (1984) found that liver mitochondria isolated from rats exhaustively exercised and/or starved, had lower P/O rations and this effect could be attributed to increased fatty acids by a number of criteria, including reversal of the effects by incubating the mitochondria with bovine serum albumin. Stimulation of isolated white adipose tissue cells by β-adrenergic agonists has been attributed to uncoupling by fatty acids released from endogenous triacylglycerols (Davis & Martin, 1982). In general it is not easy to detect whether physiological changes in fatty acids increase the mitochondrial proton leak, as the fatty acids also act as respiratory substrates and can stimulate ATP consumption (see Schonfeld *et al.*, 1988; Nobes *et al.*, 1990*b*).

In brown adipose tissue, the mitochondria have a specialized protein mediating a proton leak (reviewed in Nicholls & Locke, 1984). In the unstimulated state this leak is blocked by nucleotide binding to the uncoupling protein. Stimulation of the cells by noradrenaline results in fatty acid release from endogenous triacylglycerols, and these fatty acids can displace the nucleotides from the uncoupling protein resulting in a considerable stimulation of respiration and fatty acid oxidation (Nicholls & Locke, 1984). In adult rats in 'normal' conditions the uncoupling protein is found only in brown adipose tissue; however, recently (Shinohara *et al.*, 1991) the mRNA for the uncoupling protein has been found in the liver of new-born rats and adult rats when cold-stressed. The leak characteristics of rat liver mitochondria change dramatically during the first hours after birth (Valcarce *et al.*, 1990), suggesting the loss of expression of the uncoupling protein during development.

Shuttling and channelling of ATP

ATP must diffuse from mitochondria to its site of utilization in the cytosol or plasma membrane, and ADP must diffuse in the opposite direction. Whether this diffusion ever limits ATP turnover is controversial (reviewed in Jones, 1986). The creatine/phosphocreatine couple has been proposed to act as a shuttle for ATP as follows: mitochondrial-bound creatine kinase uses ATP to phosphorylate creatine at the mitochondria, the phosphocreatine diffuses to other isoenzymes of creatine kinase in the cytosol and plasma membrane, which then use the phosphocreatine to phosphorylate locally generated ADP, the creatine then diffuses back to the mitochondria (see Bessman & Carpenter, 1985). Because the ratio of [ATP]/[ADP] measured *in vivo* is so high (about 100:1) it seems unlikely that ATP diffusion limits energy metabolism. However, the lower concentration of ADP might limit turnover so that in the absence of the shuttle a much lower [ATP]/[ADP] ratio might be required to maintain turnover rates. Thus, the phosphocreatine shuttle may have evolved in skeletal muscle, heart and neurons partly to maintain a high ATP/ADP ratio. Substantial depletion of creatine, creatine kinase or creatine kinase activity has been found to reduce contractile function in the heart (Zweier *et al.*, 1991).

Recently it has been shown that the mitochondrial creatine kinase is localized to contact sites between the inner and outer mitochondrial membrane (Biermans *et al.*, 1989, 1990), and that the creatine kinase may have preferential access to mitochondrially generated ATP relative to extramitochondrial ATP (Erickson-Viitanaen *et al.*, 1982; Brooks & Suelter, 1987; Kuznetsov *et al.*, 1989). Creatine kinase can specifically induce contact sites (Rojo *et al.*, 1991), the contact sites appear to affect the activity of creatine kinase (Erickson-Viitanaen *et al.*, 1982; Brooks & Suelter, 1987) and the frequency of contact sites varies with metabolic state (Biermans *et al.*, 1989, 1990). Similarly, hexokinase isoenzyme 1 has been found to be mainly located at contact sites (Wieler *et al.*, 1985) and has preferential access to mitochondrially generated ATP relative to extramitochondrial ATP (Arora & Pendersen, 1988), but is bound to the outer membrane pore protein, porin (Fiek *et al.*, 1982). Hexokinase is activated by binding to mitochondria, and binding depends on frequency of contact sites and on metabolic state (binding increased by adrenaline and decreased by glucose or fatty acids in liver) (see Brdiczka *et al.*, 1990; Klug *et al.*, 1984). These findings have led to the concept of contact sites as a microcompartment where creatine kinase and hexokinase (and glycerol kinase) have preferential access to ATP exported by the adenine nucleotide carrier, and the efficiency of this microcompartment is regulated by a variety of metabolic signals.

Control by moiety pool size

Energy metabolism largely consists of a number of coupled moiety conserved cycles e.g. NADH/NAD^+ and ATP/ADP. In the short term we have seen that these ratios can control rates. In the medium and long term, rates may also be controlled by the biosynthetic and degradative pathways controlling the level of the moieties e.g. NAD^++NADH and ATP+ADP. Relatively little is known about this type of control, although it is of some medical interest as some vitamins are precursors for the coenzymes. Significant control is thought to be exerted in some conditions by total intramitochondrial adenine nucleotides (Aprille, 1988), cell phosphate (Erecinska *et al.*, 1977; Bygrave *et al.*, 1990), extramitochondrial guanine nucleotides (Cohen *et al.*, 1981), mitochondrial ubiquinone (Lenaz & Fato, 1986), Krebs cycle intermediates (Lee & Davis, 1979; Sahlin *et al.*, 1990), and possibly cell carnitine and CoA levels (Bremer, 1983; Bremer & Osmundsen, 1984).

WHAT DOES CONTROL MITOCHONDRIAL RESPIRATION AND ATP SYNTHESIS?

Evidence *in vivo*

Which control mechanisms operate to cause changes in respiration and ATP synthesis during physiological transitions? The mechanisms by which increased work load elicits increased respiration in heart has been actively studied (reviewed by Heineman & Balaban, 1990). ^{31}P-n.m.r. of free ATP, phosphocreatine and P_i have shown that these metabolites (and the estimated [ADP] and phosphorylation potential) do not change significantly over a wide range of work loads in perfused and *in situ* heart. Various experimental conditions in perfused heart can cause the appearance of correlations between work rate, respiration and estimated ADP level, including high pyruvate or acetate concentrations, ischaemia, KCl arrest, and the blockage of calcium transport across the mitochondrial inner membrane by Ruthenium Red (see Heineman & Balaban, 1990). The latter finding suggests that the increased respiration is caused by increased mitochondrial calcium, which might stimulate matrix dehydrogenases, the respiratory chain and/or the ATP synthase. Measurements of NADH level by surface fluorescence or enzyme equilibrium methods have mostly shown a decrease in the mitochondrial NADH/NAD^+ ratio with increased work load (Williamson *et al.*, 1976; Kobayashi & Neely,

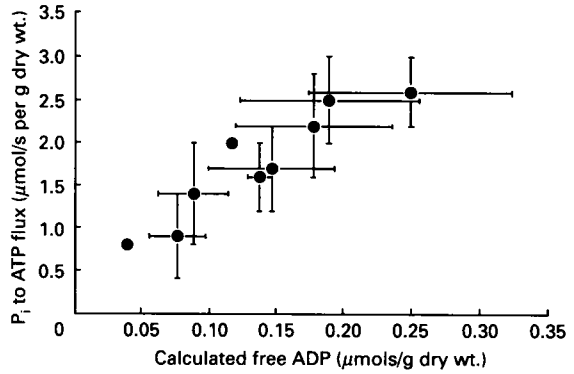

Fig. 7. Relation between estimated ADP concentration and the rate of ATP turnover in the rat hind limb *in vivo* with variable work load

ADP was estimated from phosphocreatine, ATP and P_i levels, and ATP turnover was estimated by saturation energy transfer, using ^{31}P-n.m.r. Reprinted with permission from Brindle *et al.* (1989), © (1989) American Chemical Society.

1979; Nishiki *et al.*, 1978), but a more recent study has shown an increase in NADH fluorescence with moderate increases in work rate (Katz *et al.*, 1987). Thus the mechanism of respiratory control by work load in heart is unclear at the moment, but may involve mitochondrial calcium. In smooth muscle also contraction causes no significant change in estimated [ADP], but there is no evidence for or against mitochondrial calcium being involved in respiratory control in this muscle type (Paul, 1989).

In skeletal muscle changes in respiration with work load are accompanied by changes in adenine nucleotides measured by ^{31}P-n.m.r. which are probably sufficient to account for the respiratory changes by a simple substrate/product effect (Kushmerick & Meyer, 1985; Taylor *et al.*, 1986). Brindle *et al.* (1989) found a proportional relation between free [ADP] (measured by ^{31}P-n.m.r.) and ATP turnover (measured by saturation energy transfer) in rat hind limb *in vivo* with increased work load (Fig. 7). In liver, metabolism is relatively constant but may increase due to increased substrates for gluconeogenesis and urea synthesis. These substrates also act as respiratory substrates, thus stimulating both ATP production and utilization (Letko *et al.*, 1983; Letko & Halangk, 1986; Brown *et al.*, 1990*c*). In other organs the dominant mechanism of regulation of energy metabolism is unclear.

Summary and conclusions

We have seen that there is no simple answer to the question 'what controls respiration?' The answer varies with (*a*) the size of the system examined (mitochondria, cell or organ), (*b*) the conditions (rate of ATP use, level of hormonal stimulation), and (*c*) the particular organ examined. Of the various theories of control of respiration outlined in the introduction the ideas of Chance & Williams (1955, 1956) give the basic mechanism of how respiration is regulated. Increased ATP usage can cause increased respiration and ATP synthesis by mass action in all the main tissues. Superimposed on this basic mechanism is calcium control of matrix dehydrogenases (at least in heart and liver), and possibly also of the respiratory chain (at least in liver) and ATP synthase (at least in heart). In many tissues calcium also stimulates ATP usage directly; thus calcium may stimulate energy metabolism at (at least) four possible sites, the importance of each regulation varying with tissue. Regulation of multiple sites may occur (from a teleological point of view) because: (*a*) energy metabolism is branched and thus proportionate regulation of branches is required in order to maintain constant fluxes to branches (e.g. to proton leak or different ATP uses); and/or (*b*) control over fluxes is shared by a number of reactions, so that large increases in flux requires stimulation at multiple sites because each site has relatively little control. Control may be distributed throughout energy metabolism, possibly due to the necessity of minimizing cell protein levels (see Brown, 1991).

The idea that energy metabolism is regulated by energy charge (as proposed by Atkinson, 1968, 1977) is misleading in mammals. Neither mitochondrial ATP synthesis nor cellular ATP usage is a unique function of energy charge as AMP is not a significant regulator (see for example Erecinska *et al.*, 1977). The near-equilibrium hypothesis of Klingenberg (1961) and Erecinska & Wilson (1982) is partially correct in that oxidative phosphorylation is often close to equilibrium (apart from cytochrome oxidase) and as a consequence respiration and ATP synthesis are mainly regulated by (*a*) the phosphorylation potential, and (*b*) the NADH/NAD^+ ratio. However, oxidative phosphorylation is not always close to equilibrium, at least in isolated mitochondria, and relative proximity to equilibrium does not prevent the respiratory chain, the proton leak, the ATP synthase and ANC having significant control over the fluxes. Thus in some conditions respiration rate correlates better with [ADP] than with phosphorylation potential, and may be relatively insensitive to mitochondrial NADH/NAD^+ ratio.

In addition to control by ATP utilization and calcium mobilization there are various specialized controls in different tissues: (*a*) control by respiratory substrate supply (including fatty acids), (*b*) control by oxygen supply, and (*c*) possible shuttling of ATP by creatine kinase and hexokinase. In the medium term rates may be altered by changes in coenzyme and moiety pool levels. In the long term changes in protein levels by gene expression occur.

I thank the Royal Society, Welcome Trust and St. Catherines College, Cambridge, for financial support.

REFERENCES

Akerboom, T. P. M., Bookelman, H. & Tager, J. M. (1977) FEBS Lett. **74**, 50–54
Andrews, S. B., Leapman, R. D., Landis, D. M. D. & Reese, T. S. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 1713–1717
Aprille, J. R. (1988) FASEB J. **2**, 2547–2556
Arora, K. K. & Pendersen, P. L. (1988) J. Biol. Chem. **263**, 17422–17428
Ashcroft, F. M. (1988) Annu. Rev. Neurosci, **11**, 97–118
Astrand, P. O. (1989) in Energy Transformation in Cells and Organisms (Wieser, W. & Gnaiger, E., eds.), pp. 254–261, Thieme, Stuttgart, New York
Atkinson, D. E. (1968) Biochemistry **7**, 4030–4034
Atkinson, D. E. (1977) Cellular Energy Metabolism and Its Regulation, Academic Press, New York
Balaban, R. S. & Mandel, L. J. (1988) Am. J. Physiol. **254**, F407–F416
Bauer, C. & Kurtz, A. (1989) Annu. Rev. Physiol. **51**, 845–856
Beck, J. S., Breton, S., Mairbaurl, H., Laprade, R. & Giebisch, G. (1991) Am. J. Physiol. **261**, F634–F639
Bessman, S. P. & Carpenter, C. L. (1985) Annu. Rev. Biochem. **54**, 831–862
Biermans, W., Bernaert, I., De Bie, M., Nys, B. & Jacob, W. (1989) Biochem. Biophys. Acta **974**, 74–80
Biermans, W., Bakker, A. & Jacobs, W. (1990) Biochim. Biophys. Acta **1018**, 225–228
Bohnensak, R., Bohme, G., Kuster, U., Letko, G. & Schonfeld, P. (1981) Arch. Biochem. Biophys. **209**, 219–229
Borst, P., Loos, J. A., Crist, E. J. & Slater, E. C. (1962) Biochim. Biophys. Acta **62**, 509–518
Brand, M. D. (1990) Biochim. Biophys. Acta **1018**, 128–133
Brand, M. D. & Murphy, M. P. (1987) Biol. Rev. **62**, 141–193
Brand, M. D., Hafner, R. P. & Brown, G. C. (1988) Biochem. J. **225**, 535–539
Brand, M. D., D'Alessandri, L., Reis, H. M. G. P. U. & Hafner, R. P. (1990) Arch. Biochem. Biophys. **283**, 278–284

Brdiczka, D., Bucheler, K., Kottke, M., Adams, V. & Nalam, V. K. (1990) Biochim. Biophys. Acta **1018**, 234–238
Bremer, J. (1983) Physiol. Rev. **63**, 1420–1480
Bremer, J. & Osmundsen, H. (1984) in Fatty Acid Metabolism and its Regulation (Numa, S., ed.), pp. 113–154, Elsevier, Amsterdam
Brindle, K. M., Blackledge, M. J., Challis, R. A. J. & Radda, G. K. (1989) Biochemistry **28**, 4887–4893
Brooks, S. P. J. & Suelter, C. H. (1987) Arch. Biochem. Biophys. **257**, 144–153
Brown, G. C. (1989) J. Biol. Chem. **264**, 14704–14709
Brown, G. C. (1991) J. Theor. Biol. **153**, 195–203
Brown, G. C. & Brand, M. D. (1985) Biochem. J. **225**, 399–405
Brown, G. C. & Brand, M. D. (1986) Biochem. J. **234**, 75–81
Brown, G. C. & Brand, M. D. (1988) Biochem. J. **252**, 473–479
Brown, G. C. & Brand, M. D. (1991) Biochim. Biophys. Acta **1059**, 55–62
Brown, G. C., Hafner, R. P. & Brand, M. D. (1990*a*) Eur. J. Biochem. **188**, 321–325
Brown, G. C., Hafner, R. P. & Brand, M. D. (1990*b*) in Monovalent Cations in Biological Systems (Pasternak, C. A., ed.), pp. 237–251, CRC Press, Boca Raton, FL
Brown, G. C., Lakin-Thomas, P. L. & Brand, M. D. (1990*c*) Eur. J. Biochem. **192**, 355–362
Bygrave, F. L., Lenton, L., Altin, J., Setchell, B. A. & Karjalainen, A. (1990) Biochem. J. **267**, 69–73
Capitanio, N., De Nitto, E., Villani, G., Capitanio, G. & Papa, S. (1990) Biochemistry **29**, 2939–2945
Chance, B. & Williams, G. R. (1955) J. Biol. Chem. **217**, 383–393
Chance, B. & Williams, G. R. (1956) Adv. Enzymol. **17**, 65–134
Chance, B., Leigh, J. S., Clark, B. J., Maris, J., Kent, J., Nioka, S. & Smith, D. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 8384–8388
Chazotte, B. & Hackenbrock, C. R. (1988*a*) J. Biol. Chem. **263**, 14359–14367
Chazotte, B. & Hackenbrock, C. R. (1988*b*) in Integration of Mitochondrial Function (Lemasters, J. J., Hackenbrock, C. R., Thurman, R. G. & Westerhoff, H. V., eds.), pp. 53–61, Plenum Press, New York
Chazotte, B. & Hackenbrock, C. R. (1989) J. Biol. Chem. **264**, 4978–4985
Chernyak, B. V. & Koslov, I. A. (1986) Trends Biochem. Sci. **11**, 32–36
Clausen, T., Van Hardeveld, C. & Everts, M. E. (1991) Physiol. Rev. **71**, 733–774
Cohen, M. B., Maybaurn, J. & Sadee, W. (1981) J. Biol. Chem. **256**, 8713–8717
Cooke, R. & Pate, E. (1985) Biophys. J. **48**, 789–798
Cooper, C. E. (1990) Biochim. Biophys. Acta **1017**, 187–203
Corkey, B. E., Tornheim, K., Deeney, J. T., Glennon, M. C., Parker, J. C., Matschinsky, F. M., Ruderman, N. B. & Prentki, M. (1988) in Integration of Mitochondrial Function (Lemasters, J. J., Hackenbrock, C. R., Thurman, R. G. & Westerhoff, H. V., eds.), pp. 543–550, Plenum Press, New York
Cornish-Bowden, A. & Cardenas, M. L. (1990) Control of Metabolic Processes, Plenum Press, New York
Crompton, M. (1985) Curr. Top. Membr. Transp. **25**, 231–276
Dagani, F. & Erecinska, M. (1987) J. Neurochem. **49**, 1229–1240
Dagani, F., Feletti, F. & Canevari, L. (1989) J. Neurochem. **53**, 1379–1389
Das, A. M. & Harris, D. A. (1989) FEBS Lett. **256**, 97–100
Das, A. M. & Harris, D. A. (1990) Cardiovasc. Res. **24**, 411–417
Daut, J. (1987) Biochim. Biophys. Acta **895**, 41–62
Davidson, A. M. & Halestrap, A. P. (1987) Biochem. J. **246**, 715–723
Davidson, A. M. & Halestrap, A. P. (1988) Biochem. J. **254**, 379–384
Davidson, A. M. & Halestrap, A. P. (1989) Biochem. J. **258**, 817–821
Davis, E. J. & Davis-Van Thienen, W. I. A. (1978) Biochem. Biophys. Res. Commun. **83**, 1260–1266
Davis, E. J. & Davis-Van Thienen, W. I. A. (1984) Arch. Biochem. Biophys. **233**, 573–581
Davis, E. J. & Lumeng, L. (1975) J. Biol. Chem. **250**, 2275–2282
Davis, R. J. & Martin, B. R. (1982) Biochem. J. **206**, 611–618
Denton, R. M. & McCormack, J. G. (1980) FEBS Lett. **119**, 1–8
Denton, R. M., McCormack, J. G. & Edgell, N. J. (1980) Biochem. J. **190**, 107–117
De Meis, L. & Sorenson, M. M. (1989) Biochim. Biophys. Acta **984**, 373–378
Doussiere, J., Ligeti, E., Brandolin, G. & Vignais, P. V. (1984) Biochim. Biophys. Acta **766**, 492–500
Duszynski, J., Groen, A. K., Wanders, R. J. A., Vervoorn, R. C. & Tager, J. M. (1982) FEBS Lett. **146**, 262–266
Edelstone, D. I., Paulone, M. E. & Holzman, I. R. (1984) Am. J. Obstet. Gynecol. **150**, 513–518
Edwards, A. D., Brown, G. C., Cope, M., Wyatt, J. S., McCormick, D. C., Roth, S. C., Delpy, D. T. & Reynolds, E. O. R. (1991) J. Appl. Physiol. **71**, 1907–1913
Erecinska, M. & Dagani, F. (1990) J. Gen. Physiol. **95**, 591–616
Erecinska, M. & Wilson, D. F. (1982) J. Membr. Biol. **70**, 1–14
Erecinska, M., Stubbs, M., Miyata, Y., Ditre, C. M. & Wilson, D. F. (1977) Biochim. Biophys. Acta **462**, 20–35
Erickson-Viitanen, S., Viitanen, P., Geiger, P. J., Yang, W. C. T. & Bessman, S. P. (1982) J. Biol. Chem. **257**, 14395–14404
Fiek, C., Benz, R., Roos, N. & Brdiczka, D. (1982) Biochim. Biophys. Acta **688**, 429–440
Ferguson-Miller, S., Rajaratham, K., Hochman, J. & Schindler, M. (1988) in Integration of Mitochondrial Function (Lemasters, J. J., Hackenbrock, C. R., Thurman, R. G. & Westerhoff, H. V., eds.), pp. 23–31, Plenum Press, New York
Forman, N. G. & Wilson, D. F. (1983) J. Biol. Chem. **258**, 8649–8655
Fredholm, B. B. & Dunwiddie, T. V. (1988) Trends Pharmacol. Sci. **9**, 130–138
Funk, C. I., Clark, A. & Connett, R. J. (1990) Am. J. Physiol. **258**, C995–C1005
Gilbert, R. J., Valente, G., Deeney, J. T. & Corkey, B. E. (1991) Am. J. Physiol. **261**, G634–G640
Groen, A. K., Wanders, R. J. A., Westerhoff, H. V., van der Meer, A. & Tager, J. M. (1982) J. Biol. Chem. **257**, 2754–2757
Gronostajski, R. M., Pardee, A. B. & Goldberg, A. L. (1985) J. Biol. Chem. **260**, 3344–3349
Gutknecht, J. (1988) J. Membr. Biol. **106**, 83–93
Gyulai, L., Roth, Z., Leigh, J. S., Jr. & Chance, B. (1985) J. Biol. Chem. **260**, 3947–3954
Hackenbrock, C. R. & Gupte, S. S. (1988) in Integration of Mitochondrial Function (Lemasters, J. J., Hackenbrock, C. R., Thurman, R. G. & Westerhoff, H. V., eds.), pp. 15–22, Plenum Press, New York
Hafner, R. P., Brown, G. C. & Brand, M. D. (1990*a*) Biochem. J. **265**, 731–734
Hafner, R. P., Brown, G. C. & Brand, M. D. (1990*b*) Eur. J. Biochem. **188**, 313–319
Halestrap, A. P. (1987) Biochem. J. **244**, 159–164
Halestrap, A. P. (1989) Biochim. Biophys. Acta **973**, 355–382
Hampson, N. B., Camporesi, E. M., Stolp, B. W., Moon, R. E., Shook, J. E., Griebel, J. A. & Piantodosi, C. A. (1990) J. Appl. Physiol. **69**, 907–913
Hansford, R. G. (1980) Curr. Top. Bioenerg. **10**, 217–278
Hansford, R. G. (1985) Rev. Physiol. Biochem. Pharmacol. **102**, 1–72
Hansford, R. G. & Johnson, R. N. (1975) J. Biol. Chem. **250**, 8361–8375
Hansford, R. G., Moreno-Sanchez, R. & Staddon, J. (1988) in Integration of Mitochondrial Function (Lemasters, J. J., Hackenbrock, C. R., Thurman, R. G. & Westerhoff, H. V., eds.), pp. 235–244, Plenum Press, New York
Heineman, F. W. & Balaban, R. S. (1990) Annu. Rev. Physiol. **52**, 523–542
Hucul, J. A., Henshaw, E. C. & Young, D. A. (1985) J. Biol. Chem. **260**, 15585–15591
Ikehara, T., Yamaguchi, H., Hosokawa, K., Sakai, T. & Miyamoto, H. (1984) J. Gen. Physiol. **119**, 273–282
Jacobus, W. E., Moreadith, R. W. & Vandegaer, K. M. (1982) J. Biol. Chem. **257**, 2397–2402
Jones, D. P. (1986) Am. J. Physiol. **250**, C663–C675
Kacser, H. & Burns, J. A. (1973) Symp. Soc. Exp. Biol. **32**, 65–104
Kadenbach, B. (1986) J. Bioenerg. Biomembr. **18**, 39–54
Kagawa, Y. (1984) in Bioenergetics (Ernster, L., ed.), pp. 149–186, Elsevier, Amsterdam
Kammermeier, H., Schmidt, P. & Jungling, E. (1982) J. Mol. Cell. Cardiol. **14**, 267–277
Kauppinen, R. A. & Nicholls, D. G. (1986*a*) Eur. J. Biochem. **158**, 159–165
Kauppinen, R. A. & Nicholls, D. G. (1986*b*) FEBS Lett. **199**, 222–226
Katz, L. A., Kotetsky, A. P. & Balaban, R. J. (1987) FEBS Lett. **221**, 270–276
Katz, L. A., Koretsky, A. P. & Balaban, R. S. (1988) Am. J. Physiol. **255**, H185–H188
Kelly, J. M. & McBride, B. W. (1990) Proc. Nutr. Soc. **49**, 203–215
Kentish, J. C. (1986) J. Physiol. (London) **370**, 585–604
Kessler, M., Bruley, D. F., Clark, L. C., Lubbers, D. W., Silver, I. A. & Strauss, J. (1973) Oxygen Supply, University Park Press, Baltimore, MD
Kim, D. K., Heineman, F. W. & Balaban, R. J. (1991) Am. J. Physiol. **260**, H1767–H1773

Kinter, D. J. H., Fitzpatrick, J. A., Louie, J. A. & Gilboe, D. D. (1984) Am. J. Physiol. **247**, E475–E482.
Klingenberg, M. (1961) Biochem. Z. **335**, 231–272
Klingenberg, M. (1968) in Biological Oxidations (Singer, T. P., ed.), pp. 3–54, Interscience, New York
Klingenberg, M. (1985) in The Enzymes of Biological Membranes (Martinosi, A. N., ed.) Plenum Press, New York
Klug, G. A., Krause, J., Ostlund, A-K., Knoll, G. & Brdiczka, D. (1984) Biochim. Biophys. Acta **764**, 272–282
Kobayashi, K. & Neely, J. R. (1979) J. Mol. Cell. Cardiol. **15**, 369–382
Koretsky, A. P. & Balaban, R. S. (1987) Biochim. Biophys. Acta **893**, 398–408
Korzeniewski, B. & Froncisz, W. (1991) Biochim. Biophys. Acta **1060**, 210–223
Kunz, W., Bohnensak, R., Bohme, G., Kuster, U., Letko, G. & Schonfeld, P. (1981) Arch. Biochem. Biophys. **209**, 219–229
Kunz, W., Gellerich, F. N., Schild, L. & Schonfeld, P. (1988) FEBS Lett. **233**, 17–21
Kupriyanov, V. V., Lakomkin, V. L., Korchazhkina, O. V., Stepanov, V. A., Steinschneider, A. V. & Kapelko, V. I. (1991) Biochim. Biophys. Acta **1058**, 386–399
Kushmerick, M. J. & Meyer, R. A. (1985) Am. J. Physiol. **248**, C542–C549
Kuznetsov, A. V., Khuchua, Z. A., Vassil'eva, E. V., Medved'eva, N. V. & Saks, V. A. (1989) Arch. Biochem. Biophys. **268**, 176–190
Lakin-Thomas, P. L. & Brand, M. D. (1988) Biochem. J. **256**, 167–173
LaNoue, K. F., Jeffries, F. M. H. & Radda, G. K. (1986) Biochemistry **25**, 7667–7675
Lardy, H. A. & Wellman, J. (1952) J. Biol. Chem. **195**, 215–224
Latipaa, P. M., Karki, T. T., Hiltunen, J. K. & Hassinen, I. E. (1986) Biochim. Biophys. Acta **875**, 293–300
Lee, S.-H. & Davis, E. J. (1979) J. Biol. Chem. **254**, 420–430
Lenaz, G. & Fato, R. (1986) J. Bioenerg. Biomembr. **18**, 369–401
Lenaz, G., Fato, R., Castelluccio, C., Degli Esposti, M., Samworth, C. M., Battino, M. & Parenti Castelli, G. (1988) in Integration of Mitochondrial Function (Lemasters, J. J., Hackenbrock, C. R., Thurman, R. G. & Westerhoff, H. V., eds.), pp. 33–52, Plenum Press, New York
Letko, G. & Halangk, W. (1986) Biomed. Biochim. Acta **45**, 265–271
Letko, G., Kuster, U., Duszynski, J. & Kunz, W. (1980) Biochim. Biophys. Acta **593**, 196–203
Letko, G., Kuster, U. & Pohl, K. (1983) Biomed. Biochim. Acta **42**, 323–333
Ligeti, E., Brandolin, G., Dupont, Y. & Vignais, P. (1985) Biochemistry **24**, 4423–4428
Lippe, G., Sorgato, M. C. & Harris, D. A. (1988) Biochim. Biophys. Acta **933**, 12–21
Lochner, A., Van Niekerk, I. & Kotze, J. C. N. (1981) J. Mol. Cell. Cardiol. **13**, 991–997
McCormack, J. G. & Denton, R. M. (1990) Annu. Rev. Physiol. **52**, 451–466
McCormack, J. G. & England, P. J. (1983) Biochem. J. **214**, 581–585
McCormack, J. G., Browne, H. M. & Dawes, N. J. (1989) Biochim. Biophys. Acta **973**, 420–427
McCormack, J. G., Halestrap, A. P. & Denton, R. M. (1990) Physiol. Rev. **70**, 391–425
McGarry, J. D. & Foster, D. W. (1980) Annu. Rev. Biochem. **49**, 395–420
Mendelsohn, S. L., Nordeen, S. K. & Young, D. A. (1977) Biochem. Biophys. Res. Commun. **79**, 53–60
Moody, A. J., Cooper, C. E. & Rich, R. R. (1991) Biochim. Biophys. Acta **1059**, 189–207
Moreno-Sanchez, R. (1985) J. Biol. Chem. **260**, 12554–12560
Moreno-Sanchez, R., Espinosa-Garcia, M. T. & Raya, J. C. (1988) in Integration of Mitochondrial Function (Lemasters, J. J., Hackenbrock, C. R., Thurman, R. G. & Westerhoff, H. V., eds.), pp. 297–304, Plenum Press, New York
Moreno-Sanchez, R., Hogue, B. A. & Hansford, R. G. (1990) Biochem. J. **268**, 421–428
Moreno-Sanchez, R., Devars, S., Lopez-Gomez, F., Uribe, A. & Corona, N. (1991) Biochim. Biophys. Acta **1060**, 284–292
Moroney, P. M., Scholes, T. A. & Hinkle, P. C. (1984) Biochemistry **23**, 4991–4997
Murphy, M. P. (1989) Biochim. Biophys. Acta **977**, 123–141
Murphy, M. P. & Brand, M. D. (1987) Biochem. J. **243**, 499–505
Nicholls, D. G. (1974) Eur. J. Biochem. **50**, 305–315
Nicholls, D. G. & Locke, R. M. (1984) Physiol. Rev. **64**, 1–64
Nishiki, K., Erecinska, M. & Wilson, D. F. (1978) Am. J. Physiol. **234**, C73–C81
Nobes, C. D., Brown, G. C., Olive, P. N., & Brand, M. D. (1990*a*) J. Biol. Chem. **265**, 12903–12909
Nobes, C. D., Hay, W. W. & Brand, M. D. (1990*b*) J. Biol. Chem. **265**, 12910–12915
Oguro, K., Kubota, K., Kimura, T. & Hashimoto, K. (1973) Jpn. J. Pharmacol. **23**, 459–466
Oshino, N., Sugano, T., Oshino, R. & Chance, B. (1974) Biochim. Biophys, Acta **368**, 298–310
Pall, M. L. (1985) Curr. Top. Cell Regul. **25**, 1–20
Papa, S., Lorusso, M., Izzo, G. & Capuano, F. (1981) Biochem. J. **194**, 395–406
Patel, T. R., Sambasivarao, D. & Rashed, H. (1988) Arch. Biochem. Biophys. **264**, 368–375
Paul, R. J. (1989) Annu. Rev. Physiol. **51**, 331–349
Paul, R. J., Elzinga, G. & Yamada, K. (1989) Muscle Energetics, Liss, New York
Petersen, L. C., Nicholls, P. & Degn, H. (1974) Biochem. J. **142**, 247–252
Petersen, L. C., Nicholls, P. & Degn, H. (1976) Biochim. Biophys. Acta **452**, 59–65
Pullman, M. E. & Monroy, G. C. (1963) J. Biol. Chem. **238**, 3762–3769
Quinlan, P. T. & Halestrap, A. P. (1986) Biochem. J. **236**, 789–800
Rojo, M., Hovius, R., Demel., R. A., Nicolay, K. & Wallimann, T. (1991) J. Biol. Chem. **266**, 20290–20295
Rottenberg, H. & Gutman, M. (1977) Biochemistry **16**, 3220–3226
Rutter, G. A. & Denton, R. M. (1988) Biochem. J. **252**, 181–189
Saggerson, D. (1986) Biochem. Soc. Trans. **14**, 679–681
Sahlin, K., Katz, A. & Broberg, S. (1990) Am. J. Physiol. **259**, C834–C841
Schonfeld, P., Wojtczak, A. B., Geelen, M. J. H., Kunz, W. & Wojtczak, L. (1988) Biochim. Biophys. Acta **936**, 280–288
Shinohara, Y., Shima, A., Kamida, M. & Terada, H. (1991) FEBS Lett. **293**, 173–174
Siesjo, B. K. (1978) Brain Energy Metabolism, Wiley, New York
Soboll, S., Grundel, S., Schwabe, U. & Scholz, R. (1984*a*) Eur. J. Biochem. **141**, 231–236
Soboll, S., Seitz, H. J., Sies, H., Ziegler, B. & Scholz, R. (1984*b*) Biochem. J. **220**, 371–376
Soltoff, S. P. (1986) Annu. Rev. Physiol. **48**, 9–31
Soltoff, S. P. & Mandel, L. J. (1984) J. Gen. Physiol. **84**, 643–662
Somlyo, A. P., Bond, M. & Somiyo, A. V. (1985) Nature (London) **314**, 622–625
Sperelakis, N. (1988) J. Mol. Cell. Cardiol **20**, 75–105
Su, C. (1985) Annu. Rev. Physiol. **47**, 665–676
Sugden, M. C., Holness, M. J. & Palmer, T. N. (1989) Biochem. J. **263**, 313–323
Tager, J. M., Wanders, A. J. A., Groen, A. K., Kunz, W., Bohnensak, A., Kuster, V., Letko, G., Bohme, G., Duszynski, J. & Wojtczak, L. (1983) FEBS Lett. **151**, 1–9
Tamura, M., Hazeki, O., Noika, S. & Chance, B. (1989) Annu. Rev. Physiol. **51**, 813–834
Tani, M. (1990) Annu. Rev. Physiol. **52**, 543–559
Taylor, D. J., Styles, P., Mathews, P. M., Arnold, D. M. & Gadian, D. G. (1986) Magn. Reson. Med. **3**, 44–54
Tessitore, N., Sakhrani, L. M. & Massry, S. G. (1986) Am. J. Physiol. **251**, C120–C127
Unitt, J. F., McCormack, J. G., Reid, D., MacLachlan, L. K. & England, P. J. (1989) Biochem. J. **262**, 293–301
Valcarce, C., Vitorica, J., Satrustegui, J. & Cuezva, J. M. (1990) J. Biochem. (Tokyo) **108**, 642–645
van Dam, K., Westerhoff, H. V., Rutgers, M., Bode, J. A., De Jonge, P. C., Bos, M. M. & Van Den Berg, G. (1981) in Vectorial Reactions in Electron and ion Transport in Mitochondria and Bacteria (Palmieri, F., Quagliariello, Siliprandi, N. & Slater, E. C., eds.), pp. 389–397, Elsevier, Amsterdam
Vanderkooi, J. M., Erecinska, M. & Silver, I. A. (1991) Am. J. Physiol. **260**, C1131–C1150
Verhoeven, A. J., Kamer, P., Groen, A. K. & Tager, J. M. (1985) Biochem. J. **226**, 183–192
Vignais, P. V. (1976) Biochim. Biophys. Acta **456**, 1–38
Walton, G. M. & Gill, G. N. (1976) Biochim. Biophys. Acta **447**, 11–19
Waterlow, J. C. (1984) Q. J. Exp. Physiol. **69**, 409–438
Westerhoff, H. V. (1989) Biochemie **71**, 877–886
Whalen, W. J., Buerk, D. & Thuning, C. A. (1973) Am. J. Physiol. **224**, 763–768
Wieler, U., Riesinger, I., Knoll, G. & Brdiczka, D. (1985) Biochem. Med. **33**, 223–235

Willliamson, J. R., Ford, C., Illingworth, J. & Safer, B. (1976) Circ. Res. **38**, 139–148
Willis, W. T. & Dallman, P. R. (1989) Am. J. Physiol. **257**, C1080–C1085
Wilson, D. F., Owen, C. S. & Erecinska, M. (1979) Arch. Biochem. Biophys. **195**, 494–504
Wilson, D. F., Rumsey, W. L., Green T. J. & Vanderkooi, J. M. (1988) J. Biol. Chem. **263**, 2712–2718
Wittenberg, B. A. & Wittenberg, J. B. (1989) Annu. Rev. Physiol. **51**, 857–878
Yamada, E. W. & Huzel, N. J. (1988) J. Biol. Chem. **263**, 11498–11503
Zweier, J. L., Jacobus, W. E., Korecky, B. & Brandejs-Barry, Y. (1991) J. Biol. Chem. **266**, 20296–20304