THOUSANDS OF DIFFERENT METABOLITES AND ENZYMES must work together if a cell is to survive and grow. In addition to the complex ensemble of reactions within individual cells, in organisms with extensive cellular differentiation, metabolism must support the robust cell-to-cell communication needed for different cell types to contribute to integrated function of the organism. DNA contains the blueprint needed to construct the parts of the ensemble, but in living cells metabolism has to constantly and rapidly respond to changes in the environment. These metabolic responses often occur in seconds, far faster than information encoded in the DNA can be read and implemented. Real-time control of metabolism requires a metabolic unit with intrinsic properties that drive it toward a particular metabolic condition (set point) and is connected to the rest of metabolism through a network of regulatory pathways that maintain that set point (65). To control the ensemble, this central control unit needs to have sensory input from all of the individual metabolic pathways in the cell and be able to output in real time control signals appropriate to maintain stable operation of cellular metabolism as a whole. As noted earlier (65), in eukaryotes the only metabolic pathway having the requisite connections to all of cellular metabolism is oxidative phosphorylation. Oxidative phosphorylation is responsible for setting and maintaining the energy state ([ATP]/[ADP][P_i]) ratio near 10^5 M^-1. This is 5 x 10^{10} greater than the equilibrium value of 2 x 10^{-6} M^-1 (18, 36). In mammals, excursions of more than a factor of about 5 from the set point for any cell results in impaired function and can be tolerated only for limited periods of time. Thus oxidative phosphorylation not only drives the energy state ([ATP]/[ADP][P_i]) a factor of 5 x 10^{10} from equilibrium but also maintains the value (average over time) within a factor of 5.

It can readily be inferred that oxidative phosphorylation acts as the control unit for cellular metabolism, and the energy state is the determinant of metabolic homeostasis. To be sure, however, it is necessary to know the nature of the program that determines the set point for the energy state and that the requisite metabolic signaling pathways are present and functional. A survey of current literature and metabolic charts shows extensive signaling pathways, as indicated by the extensive roles of ATP and other nucleotide di-, tri-, and monophosphates and reactants and/or regulatory factors throughout metabolism. A notable exception in the metabolic charts has been the minimal role of energy metabolism in maintaining the supply of metabolites, notably oxygen, to tissues. This exception is the result of an error in interpretation of measurements of the dependence of respiratory rate on oxygen pressure (P_{O2}). Respiration in suspensions of cells and isolated mitochondria remains nearly constant until the oxygen pressure (P_{O2}) is less than about 5 torr, with the P50 of less than 1 torr (see Refs. 4, 6, 43, 50, 53, 55, 58, 74, and 76). The dependence of the respiratory rate on P_{O2} has been assumed by many to be a reliable measure of the metabolic dependence on P_{O2}. This assumption is incorrect. The respiratory rate is also dependent on the energy state and level of reduction of cytochrome c, and these regulatory parameters are responsible primarily for determining the respiratory rate under all conditions except severe hypoxia (10, 65–70, 73). In the physiological range of oxygen pressures, the respiratory rate is ~50 times more sensitive to energy state than to P_{O2} (65, 66, 69, 70, 76). Changes in the energy state compensate easily for changes in P_{O2} and result in measured P50 values being as little as one-fiftieth the metabolically relevant P50 (66, 69, 70, 76). The metabolically relevant P50, i.e., the P50 had there been no change in energy state or cytochrome c reduction, is 12 torr or slightly higher (19, 66, 67, 69, 70, 76). As a result, the dependence of oxidative phosphorylation on oxygen pressure is well suited for contributing to the control of the many signaling/control pathways responsible for regulating oxygen.
delivery to tissues (10, 42, 66, 67, 72), although much remains to be worked out concerning the mechanisms involved in this regulation. A schematic illustrating the central role of oxidative phosphorylation in cellular metabolism is shown in Fig. 1.

In the current paper, evidence that oxidative phosphorylation is responsible for setting and regulating metabolic homeostasis in higher organisms will be further developed. Cytochrome c oxidase activity determines the rate of ATP synthesis by mitochondria (10, 31, 66–70, 74, 76), and a model consistent with the dependence of the rate of oxygen reduction on cytochrome c reduction, energy state, pH, and oxygen pressure has been published (76). However, in vivo, the primary source of reducing equivalents is NADH generated by the citric acid cycle, fatty acid oxidation, and amino acid catabolism. To integrate the control of cytochrome c oxidase into cellular energy metabolism as a whole, the model for cytochrome c oxidase is extended to include the first two sites of oxidative phosphorylation, coupling it to the intramitochondrial NAD pool. The result is a model that is consistent with currently available information of the dependence of energy metabolism on the intramitochondrial NAD pool, PO2, pH, and energy state.

MATERIALS AND METHODS

The graphics program Origin (www.originlab.com) was used for preparing the figures, and the steady-state rate expression was programmed in MatLab (www.mathworks.com). The steady-state rate expression for oxygen reduction by cytochrome c oxidase and the values of the fitting parameters are taken from Wilson and Vinogradov (76). It should be noted that the data to which the steady-state rate expression was fitted were for isolated mitochondria and measured at room temperature (20–23°C), whereas most of the measurements referenced in this paper are for cells or tissue and were measured at 38–40°C. In the present paper, the effects of temperature are not addressed, but clearly a comprehensive evaluation of the temperature dependence of mitochondrial function is needed. In addition, the literature on energy metabolism in intact cells and tissues is quite limited due to the difficulty in making the necessary measurements. The most important metabolites for evaluating oxidative phosphorylation are ATP, ADP, P, creatine phosphate, and creatine. 31P-MRI can readily measure the ATP, P, and creatine phosphate but not ADP or creatine. Chemical methods can measure all of these metabolites, but metabolism has to be rapidly quenched in an attempt to stop metabolic changes, locking the metabolites into the levels immediately before quenching. This is technically difficult, and although ingenious methods have been worked out to do the quenching, there is always concern as to how much change occurred during quenching. In addition, some hydrolysis of creatine phosphate (CrP). ATP, and other phosphorylated compounds may occur during the analytical procedures. Metabolite measurements from the literature have been used where possible, but in many cases it was necessary to “fill in” the data missing from one paper with values from other papers [such as total creatine concentrations or inorganic phosphate concentration ([Pi])] to make the calculations. Where fill-in values have been used they are noted as assumed values but have been selected to represent what the author perceived as a “consensus” value consistent with more than one literature report. The concentrations of ADP and AMP presented in this paper are for the free ADP and AMP, not the total cellular concentrations. They have been calculated assuming near equilibrium of the adenylate kinase and creatine phosphokinase reactions.

The rates of mitochondrial respiration are presented as turnover numbers for cytochrome c instead of oxygen consumed per unit of weight or volume of tissue. This is because the properties of oxidative phosphorylation being discussed are applicable to all eukaryotic cells. Since cells and tissues vary widely in their content of the enzymes of oxidative phosphorylation, the activity of oxidative phosphorylation expressed per unit of tissue weight or volume is cell/tissue specific. However, the activity expressed per unit of cytochrome c or cytochrome oxidase (a + a3) is independent of cell-to-cell variability and can be compared directly among cells and tissues.

The steady-state rate expression for oxidative phosphorylation based on the mechanistic model in Fig. 2 has been derived and programmed for MatLab. The program is presented in the APPENDIX along with values of the rate and equilibrium constants obtained by fit to the data for cytochrome c oxidase (taken from Ref. 76).

RESULTS

The model for cytochrome c oxidase and the steady-state expression that includes the dependence on pH, energy state, fraction of cytochrome c reduced, and Po2 predicted by that model. In a previous paper (76), we presented a model for the mechanism of oxygen reduction by cytochrome c oxidase and derived the steady-state rate expression for that model. This steady-state rate expression was then fitted to the measured dependence of the rate of mitochondrial respiration on the level of reduction of cytochrome c, the source of reducing equiva-
Fig. 2. Combining the first 2 energy-coupling sites of oxidative phosphorylation (NADH to cytochrome c of the respiratory chain) with cytochrome c oxidase, resulting in a model for all the oxidative phosphorylation. Schematic representation of oxidative phosphorylation coupled to the energy state. There are 3 coupling sites in oxidative phosphorylation; with the rate of the reaction determined by cytochrome c oxidase (coupling site 3), which is responsible for the reduction of oxygen to water. All 3 sites are coupled to ATP synthesis through a common intermediate and for which the available energy (equivalent to the voltage difference ΔQ) is near equilibrium with the energy state. A mechanistic model for the cytochrome c oxidase reaction has been developed, and the derived steady-state rate has been fitted to experimental data from suspensions of isolated mitochondria, showing the dependence of the respiratory rate on cytochrome c reduction, pH, PO2, and energy state (76). Combination of the rate expression for coupling site 3 with near equilibrium constant for coupling sites 1 and 2 is known. The result is a steady-state rate expression for all of the oxidative phosphorylation. The values of the rate constants and equilibrium constants used to fit the experimental data are included in the Appendix.

Cytochrome c oxidase is site 3 of oxidative phosphorylation. It is responsible for the reduction of oxygen to water, and therefore, it determines the rates of oxygen consumption and of ATP synthesis. Thus, any discussion of the regulation of oxidative phosphorylation and of energy metabolism in general must focus on the mechanism and regulation of cytochrome c oxidase. On the other hand, the obtained rate expression is only for site 3, and therefore, it provides limited insight into the behavior of oxidative phosphorylation when integrated into cellular metabolism. It is necessary to combine site 3 with sites 1 and 2 to obtain an expression for the behavior of oxidative phosphorylation that includes the intramitochondrial NAD pool.

Sites 1 and 2 of oxidative phosphorylation. Sites 1 and 2 are responsible for the transfer of reducing equivalents from the intramitochondrial NAD pool to cytochrome c, the substrate for cytochrome c oxidase (Fig. 2). In the process, the available energy is coupled to the synthesis of two ATPs for each of the two reducing equivalents transferred. In vivo, most of the reducing equivalents for oxidative phosphorylation are donated by NADH. The reducing equivalents from succinate and other donors that bypass the first coupling site are few enough relative to those from NADH that they can be neglected. The reaction involved in the first two sites is typically written as

\[
\text{NADH} + 2\text{ADP} + 2\text{Pi} + 2\text{cyt c}^{+3} \rightarrow \text{NAD}^+ + \text{H}^+ + 2\text{ATP} + 2\text{cyt c}^{2+} \quad (I)
\]

This reaction is readily reversible, and NAD\(^+\) can be reduced using reducing equivalents from reduced cytochrome c when ATP is added to isolated mitochondria (13, 32, 56, 68). The reported intrachain electron transfer rates, expressed as cytochrome c turnover numbers, are \(>1,000 \text{ s}^{-1}\) (5, 17), and that is more than 100 times the net rate of electron transfer rates observed in resting cells. For these and other reasons (10, 13, 66, 68, 75), the first two sites can be considered near equilibrium under cellular conditions. The equilibrium constant for reaction shown in Eq. 1 is

\[
\text{Keq} = \left( \frac{[\text{NAD}^+][\text{NADH}]}{[\text{ATP}][\text{ADP}][\text{Pi}]} \right)^2 \times \left( \frac{[\text{cyt c}^{+3}]/[\text{cyt c}^{2+}]}{[\text{H}^+]} \right)^3 \times [\text{H}^+] \quad (2)
\]

However, the rate expression for cytochrome c oxidase has been developed for coupling to the common intermediate of oxidative phosphorylation and not to synthesis of ATP. The common intermediate has been represented as a charge separation that generates a voltage that is equal to but of opposite sign of that across the energy coupling sites. This voltage across the coupling sites is designated as ΔQ and meets the requirements for reversibility and near equilibrium without implying a particular coupling mechanism. Unlike hydrolysis of ATP, the energy available (value of ΔQ) is also not dependent on pH, and this simplifies the equations. The value of ΔQ in volts can be converted to the free energy for each of the two equivalents transferred in kcal/mol or kJ/mol by multiplying the volts by 46.18 kcal/volt and 193.2 kJ/volt, respectively. The first two sites without energy coupling can be written as

\[
\text{NADH} + 2\text{cyt c}^{+3} \rightarrow \text{NAD}^+ + \text{H}^+ + 2\text{cyt c}^{2+} \quad (3)
\]

An equilibrium constant suitable for inclusion into the rate expression for site 3 is the ratio of the forward and reverse rates constants or the free energy released in the reaction.

\[
\text{Keq} = \left( \frac{[\text{NAD}^+][\text{NADH}]}{[\text{ATP}][\text{ADP}][\text{Pi}]} \right)^2 \times \left( \frac{[\text{cyt c}^{+3}]/[\text{cyt c}^{2+}]}{[\text{H}^+]} \right)^3 \times [\text{H}^+] \quad (4)
\]

The half-reduction potentials for the NAD couple (−0.320 V) and cytochrome c (0.235 V) at pH 7.0 are different by a total of 0.555 V. Therefore, the ratio of the forward and reverse rate constants is \(10^{0.555/0.0295} \approx 6.4 \times 10^{11} \text{ M}^{-1}\). This equilibrium constant and Eq. 4 allow calculation of the fraction of cytochrome c reduced as a function of \([\text{NAD}^+]/[\text{NADH}]\), Q, and pH. Insertion into the rate expression for site 3 from Wilson and Vinogradov (76) results in an steady-state expression describing the rate of respiration as a function of the intramitochondrial \([\text{NAD}^+]/[\text{NADH}]\) ratio, the energy state (Q), cellular pH, and local (intracellular) PO2. A program for solving the steady-state rate expression, written for MatLab, is presented in the Appendix.

Calculating the cellular energy state using the steady-state rate expression. The rate and equilibrium constants used to fit the rate expression to the data for site 3 (76) are given in the Appendix. These rate and equilibrium constants have been shown to be consistent with the available measurements of the
rates of intrachain electron transfers, and the equilibrium constants are consistent with the measured half-reduction potentials for each oxidation reduction reaction in the model. As noted earlier, the measurements for site 3 (isolated mitochondria) were made at 20–23°C, whereas those for cell suspensions and tissue are typically for 36–40°C. This temperature difference would be expected to degrade the fit of the rate expression to the data, and a reasonably good fit would place a high priority on collection of mitochondrial data at 38°C. The calculated relationships of the [NAD]/[NADH] ratio, respiratory rate, energy state, and PO2 for an intracellular pH of 7.1 and PO2 of 58 torr are presented in Fig. 3.

**Fig. 3.** The calculated dependence of the respiratory rate (expressed as the turnover number for cytochrome c) on the energy state for intramitochondrial [NAD]/[NADH] ratios of 0.1, 1, 10, and 100. The steady-state rate expression was solved for the 4 different levels of intramitochondrial [NAD]/[NADH] at pH 7.1 and a PO2 of 58 torr. These 4 levels encompass the range of values found in cells and tissues. As the [NAD]/[NADH] increases (intramitochondrial NAD couple becomes more oxidized), a lower energy state is required to attain each respiratory rate.

Skeletal muscle under resting conditions. In resting skeletal muscle, the level of reduction of the intramitochondrial NAD couple has not been measured accurately, but the reported estimates are consistent with an [NAD]/[NADH] ratio near 0.1. The cytochrome c turnover number in resting muscle is low, estimated to be ∼0.5 s⁻¹ from the relative fraction of muscle tissue in the body, its contribution to the resting oxygen consumption, and the content of mitochondria (see Ref. 64). The levels of free ADP in muscle are difficult to measure, but the creatine phosphokinase reaction

\[
\text{Creatine phosphate} + \text{ADP} \rightarrow \text{ATP} + \text{creatine} \quad (5)
\]

can be used to calculate the [ATP]/[ADP] ratio (see Refs. 18, 36, and 63). For a turnover number of 0.5 s⁻¹, the model predicts that Q is near 0.310 V and the energy state near 1.4 × 10⁵ M⁻¹ (Figs. 3, 4A, and 5A). Experimentally measured values for the energy state in resting muscle include 2.7 × 10⁴ (63), 2 × 10⁵ (28), and 3 × 10⁵ M⁻¹ (11, 14, and 21, respectively, assuming that [Pi] = 3 mM). There is general agreement that the [CrP]/[Cr] ratio is 2 to 3, so the differences in the calculated energy state for resting skeletal muscle are due largely to differences in the concentrations of Pi, which range from 3 to 8 mM. The predictions of the model are within the range of reported experimental values.

**Fig. 4.** Dependence of the respiratory rate on oxygen pressure and energy state at [NAD]/[NADH] ratios of 0.1 (A) and 10 (B). For these calculations, the [NAD]/[NADH] and energy state were held constant, and the capillary PO2 was increased from 0 to 58 torr in 100 equal increments. The calculations were made for each 5-mV change in Q from 0.310 to 0.255 V, covering the metabolically relevant range of values. As the value of Q decreases, the respiratory rate (cytochrome c turnover number) increases and the P50 decreases.
The response of metabolism to increased rates of ATP utilization: exercising skeletal muscle. Initiation of exercise in muscle is accompanied by large increases in important regulatory signaling, including increased \([\text{Ca}^{2+}]\) and AMP. These help to rapidly activate glycogenolysis, glycolysis, and the citric acid cycle. Initially, the increased muscle activity relies on ATP provided by creatine phosphate (Eq. 5, but within a few contraction relaxation cycles the decreasing energy state has substantially activated oxidative phosphorylation. In muscle fibers, the respiratory rate (rate of ATP synthesis by oxidative phosphorylation) increases sufficiently to match the rate of ATP utilization (see also Refs. 10 and 52), except for any increase in ATP synthesis through the production of lactate. The activation of glycogenolysis, glycolysis, and the citric acid cycle is sufficient to maintain the intramitochondrial \([\text{NAD}^+] / [\text{NADH}]\) ratio despite the increased demand for ATP. Figures 3, 4A, and 5A show the behavior of energy metabolism in myocytes predicted by the model, assuming the intramitochondrial \([\text{NAD}^+] / [\text{NADH}]\) ratio is constant at a value of 0.1, as the workload increases. When muscle is relaxed (resting) the respiratory rate is low, ~0.5 s\(^{-1}\), and the predicted energy state is high, between 1 \(\times 10^5\) and 4 \(\times 10^5\) M\(^{-1}\). The cytochrome \(c\) turnover numbers at maximal work rates in tissue are similar to the maximal rates observed for isolated mitochondria from animals (see Ref. 54) and plants (8, 30, 35, 56) and are near 80 s\(^{-1}\). In the mitochondrial experiments the cytochrome \(c\) was reduced to ~30%, and extrapolation to 100% reduction would give a rate near 200 s\(^{-1}\) (66, 69, 70, 76).

Experimentally, \([\text{CrP}]\) has been reported to decrease by 11–30% in moderate exercise (11, 14, 21, 28, 51, 60) and by 25–40% in heavy exercise (see Refs. 2, 28, and 60) with no measurable decrease in [ATP]. The experimental heavy exercise conditions were not quite maximal to obtain steady states short of progression to exhaustion, where reproducible measurements are very difficult. To compare the experimental measurements with the behavior predicted by the model (Figs. 3, 4A, and 5A), we have assumed resting muscle concentrations of CrP, Cr, Pi, and ATP of 35, 12, 3.3, and 10 mM, respectively, as measured by \(^{31}\text{P}-\text{MRI}\) and calculated for the intracellular water volume (49). Assuming the intramitochondrial \([\text{NAD}] / [\text{NADH}]\) ratio remains near 0.1 and the oxygen pressure is near 30 torr, the model predicts an energy state of 3 \(\times\) 10\(^4\) M\(^{-1}\) (\([\text{CrP}]\) decrease 15%) for a turnover number of 20 s\(^{-1}\) (moderate exercise) and 1 \(\times\) 10\(^5\) M\(^{-1}\) (\([\text{CrP}]\) decrease 34%) for a turnover number of 40 s\(^{-1}\) (heavy exercise). There is no significant effect (<1%) of moderate exercise on [ATP], and even in heavy exercise the concentration of ATP decreases by only 1%. The predictions of the model are in good agreement with the experimental measurements.

Heart muscle. Metabolism of the heart has been studied extensively both as an isolated perfused organ and in vivo. Nishiki et al. (41) and Hassinen and Hiltunen (25) studied the isolated perfused rat heart, including when arrested using 20 mM KCl or \(\text{Ca}^{2+}\) free perfusate. The cytochrome \(c\) turnover was 3.9 and 5.9 s\(^{-1}\) and the corresponding energy states 1.45 \(\times\) 10\(^5\) and 7.28 \(\times\) 10\(^5\) M\(^{-1}\), respectively. When the perfusion pressure was raised to increase the metabolic rate to cytochrome \(c\) turnover of 13.9 and 28 s\(^{-1}\), the measured energy state decreased to 6.8 \(\times\) 10\(^4\) and 2.5 \(\times\) 10\(^4\) M\(^{-1}\), respectively. Chacko et al. (3) measured heart metabolism in vivo by \(^{31}\text{P}-\text{MRI}\) and reported that for both murine and human heart the energy state was similar and near 1.6 \(\times\) 10\(^5\) M\(^{-1}\). For comparison with prediction by the model, it was assumed that the intramitochondrial \([\text{NAD}^+] / [\text{NADH}]\) was 0.1, the PO2 was 30 torr, and the turnover number was 12 s\(^{-1}\). For these conditions, the model predicts an energy state near 6 \(\times\) 10\(^5\) M\(^{-1}\). The intramitochondrial \([\text{NAD}^+] / [\text{NADH}]\) ratio has been estimated for isolated perfused rat heart (25, 41) to be near 1, assuming equilibrium of glutamate dehydrogenase. This value was considered by these authors to be only approximate due to experimental limitations. It can be concluded that the experimental data available for energy metabolism in the heart are consistent with those measured in skeletal muscle as well as with the behavior predicted by the model.

Brain. The intramitochondrial \([\text{NAD}^+] / [\text{NADH}]\) for brain tissue is not known due to the lack of methods for measuring
it. It is reasonable to assume that the ratio is between 0.1 and 1 from what is known about neuronal and glial metabolism. Neurons and other brain cells contain creatine phosphokinase, and as in muscle, this reaction is generally assumed to be near equilibrium. Experimental determinations of the energy state include those of $3 \times 10^4$ M$^{-1}$ by Veech et al. (63) and $7.9 \times 10^4$ M$^{-1}$ by Nioka et al. (40). The prediction of the model, assuming a cytochrome c turnover number of $6 \text{s}^{-1}$, an [NAD$^+$/NADH] ratio near 1, and a Po$_2$ of near 30 torr, is an energy state of between 3 and $6 \times 10^4$ M$^{-1}$. Again, there is good agreement between the predictions of the model and the experimental measurements.

Liver. In liver, the intramitochondrial NAD pool has a potential of $-0.260$ to $-0.290$ V when measured by assuming near equilibrium with the $\beta$-hydroxybutyrate/acetocetate couple (75), corresponding to a free [NAD$^+$]/[NADH] ratio of 10–100. The cytochrome c turnover number in liver and isolated hepatocytes is near $6 \text{s}^{-1}$ (75). When these values are put into the steady-state rate expression for the model, the calculated energy state is $1–2 \times 10^4$ M$^{-1}$ (see Figs. 4B and 5B). Experimentally measured values for [ATP]/[ADP][Pi] include $1.6 \times 10^2$ M$^{-1}$ by Veech et al. (63).

Isolated mitochondria from heart and brain. The respiratory control ratio in isolated mitochondria is typically <10, too low to allow evaluation of physiologically relevant control of respiration, the transition region between states 3 and 4. However, mitochondria with higher respiratory control ratios, between 30 and 50, have been isolated from heart, and the dependence of respiratory rate in the control region on the concentrations of ATP, ADP, and Pi has been measured (see Refs. 27 and 73). The respiratory rate is reported to be dependent on the [ATP]/[ADP][Pi] and not the individual metabolite concentrations. Decreasing the energy state from $4.46 \times 10^6$ to $1.1 \times 10^4$ M$^{-1}$ (adjusted to 1 mM [Mg$^{2+}$]) resulted in an increase in the respiratory rate by 20-fold. The mitochondrial substrate was a combination of 5 mM each of glutamate and malate. This would be expected to generate a highly reduced intramitochondrial NAD pool {[NAD$^+$]/[NADH] < 0.1} when the rate is low (energy state is maximal). In isolated mitochondria, in contrast to mitochondria in vivo, many of the mechanisms for increasing citric acid cycle activity are missing. The model would be consistent with the data if the [NAD$^+$]/[NADH] increased from near 0.05 to near 5 as the turnover number increased. Extensive oxidation of the NAD couple in isolated mitochondria has been reported to occur for the transition from state 4 (no ADP) to state 3 (+ADP), but this has not been quantified (see Ref. 6). As a result, the predictions of the model may be considered qualitatively consistent with the experimental data, but quantitative comparison is not possible.

DISCUSSION

A model for oxidative phosphorylation has been developed by combining the steady-state rate expression for cytochrome c oxidase with the equilibrium expression for the first two sites of oxidative phosphorylation. The resulting expression describes the dependence of the rate of respiration and thereby of ATP synthesis on intramitochondrial [NAD$^+$]/[NADH], cytoplasmic energy state, Po$_2$, and pH in the mitochondrial microenvironment. Comparison of the calculated values with the experimental measurements reported in the literature shows that the predictions are within the range of experimental values; i.e., the fit is as good as can be expected. This appears to be true for oxidative phosphorylation in both animals and plants. Although mitochondria from different sources vary widely their content of enzymes other than those of oxidative phosphorylation, the respiratory chain and the control of oxidative phosphorylation are remarkably similar.

Some of the general observations on energy metabolism are reinforced by the experimental data and the predictions of the model. The creatine phosphokinase reaction is critical to the performance of muscle and other tissues that operate at high-energy states and over wide ranges of activity. In addition to the well-recognized role of creatine phosphate as an energy buffer, in tissues at high-energy states the level of ADP is very low, i.e., <1% of the levels of ATP and Pi. At these ADP levels, diffusion of ADP from the site of ATP utilization to the mitochondria is too slow to support the observed high metabolic rates. Creatine is a small molecule present at 1,000 times the concentration of ADP. It effectively increases the diffusional flux of ADP to values similar to those of ATP (+CrP) and Pi.

Creatine phosphokinase increases the importance of Pi in the regulation of energy metabolism. As noted earlier, the decrease in energy state associated with a 15% decrease in creatine phosphate during moderate exercise involves a <1% decrease in [ATP], a 1.7-fold increase in [ADP], a 1.4-fold increase in [Cr], and a 2.6-fold increase in [Pi]. In normal skeletal muscle, exercising up to moderate work rates, the rate of oxidative phosphorylation is more affected by changes in [Pi] than by changes in [ADP]. Even for strenuous exercise sufficient to produce a 30% decrease in creatine phosphate, the ATP decrease is <1%, whereas [ADP], [Cr], and [Pi] increase by 2.7-, 1.9-, and 4.2-fold, respectively. At near-maximal exercise sufficient to decrease [CrP] by 50% the [ATP] decreases by only 1%, whereas [ADP], [Cr], and [Pi] increase 4.9-, 2.5-, and 6.4-fold, respectively.

For regulatory effects other than direct control of the rate of respiration, the 4.9-fold increase in [ADP] in heavy exercise would be accompanied by an increase of 24-fold in free [AMP]. This large increase in [AMP] occurs despite [ATP] remaining essentially unchanged. Throughout the range of normal exercise, the [Pi] is a major factor in controlling the rate of oxidative phosphorylation. In addition, ADP, Pi, and AMP, particularly AMP, have important roles in regulating enzyme activities, protein synthesis, and gene expression (7, 20, 39, 52, 61).

Control of oxidative phosphorylation has a wide dynamic range and is capable of precisely meeting cellular demand for ATP synthesis that can vary by ±100-fold. The efforts of many, including Matheau-Costello and colleagues (37, 38) and Poole and colleagues (44, 45), have established that in the vascular system the capillary surface area is correlated directly with the mitochondrial content and maximal demand for oxidative phosphorylation. Almost everything else related to the role of oxygen is controversial, with widely differing opinions expressed in the literature as to the oxygen dependence of oxidative phosphorylation as well as the oxygen pressure in the cellular and mitochondrial environments.

Oxygen dependence of mitochondrial oxidative phosphorylation. With the introduction of oxygen electrodes it became possible
to measure the oxygen pressure dependence of the respiration by suspensions of mitochondria and cells. These measurements showed that the respiratory rate is independent of oxygen pressure until the concentration falls below ~5 torr, with a P50 of ~1 torr, depending on the experimental conditions (4, 6, 50, 52, 66, 69, 70, 76). Unfortunately, many workers have made critical and erroneous assumptions in interpreting the data (see Refs. 4, 6, 26, 43, 53, and 58), such as 1) that the dependence on oxygen pressure follows Michaelis kinetics and 2) that the oxygen dependence of the respiratory rate is equivalent to the dependence of metabolism on PO2. The first assumption, of Michaelis kinetics, misrepresents the complex mechanism of cytochrome c oxidase by assuming that the P50 for oxygen is determined largely by binding of oxygen to the active site. In reality, the P50 is a kinetically determined variable. The second assumption, that the P50 is representative of the metabolic dependence on PO2, is actually a composite of several erroneous assumptions. One assumption is that there are no other substrates or regulatory factors that substantively influence or are influenced by the rate of oxygen consumption; this is not true. 1) Cytochrome c oxidase reaction activity is strongly dependent on the energy state (10, 50, 52, 66, 68–70, 74–76), and this dependence has a wide dynamic range; and 2) cytochrome c oxidase activity is dependent on the level of the reduction of cytochrome c (10, 66, 69, 70, 76). The dependence of the oxidase activity on these two metabolic variables is, under physiological conditions, much greater than that on PO2.

Another assumption is that, under physiological conditions, the rate of oxygen consumption in cells and mitochondria is determined by PO2. Actually, the rate of respiration is determined by the rate of ATP utilization, and the rate of ATP utilization is not very, if at all, dependent on oxygen pressure (except under severe hypoxia). Decreasing oxygen leads not to decreasing respiratory rate but to decreasing energy state. Figure 6, A and B, shows the change in energy state needed to maintain a constant respiratory rate for different [NAD+] /[NADH] ratios and cytochrome c turnover numbers.

When energy state and cytochrome c reduction are taken into consideration, metabolic response to changing oxygen pressure is seen throughout the physiological range of oxygen pressures, i.e., to >35 torr (10, 62, 66, 69, 70, 76), and the metabolically relevant P50 is near 12 torr.

Oxygen pressure in tissues. There has been an evolution of the reported oxygen pressures in tissue due in part to improved methods of anesthesia. Early reports indicated that a substantial fraction of the tissue volume had oxygen pressures of <10 torr, sometimes even <5 torr, but more recent reports (1, 22, 34, 71) conclude that in resting muscle and in kidney the tissues have very little or no volumes with a PO2 of <10–15 torr. The mean oxygen pressures in the microcirculation are near the mixed venous value of 35–45 torr. Measurements of oxygen pressure in the interstitial space show a distribution similar to that in the microcirculation, but with a slightly lower mean oxygen pressure, presumably because of the absence of the arteriolar contribution to the microcirculation (71). No evidence was found on the lower oxygen sides of the distributions for a difference between the microcirculation and the interstitial space (difference <1 torr) in unstimulated muscle.

Logically, the difference in PO2 from the blood plasma in the capillaries to the mitochondria cannot exceed 1 torr in tissues such as liver, kidney, and brain under normal metabolic conditions. Gradients of this size or larger would result in substantial volumes of anoxia in the tissues before the tissues reached their maximal respiratory rates. This is most obvious in muscle, where the resting respiratory rates are <1% of the maximal rates. A diffusion gradient of 1 torr in resting muscle would become 100 torr at maximal rates. The diffusion gradients of several torr reported to occur in tissues under resting or low work rates are the result of methodological limitations and/or errors in interpretation. Takakura et al. (59), for example, reported oxygen diffusion gradients of 9.8 torr in resting rat skeletal muscle. The experimental tissue was saline-perfused rat hindlimb, and the intracellular oxygen pressure was calculated from the fraction saturation of myoglobin, as measured by near-infrared spectroscopy. In the physiological range

Fig. 6. Energy state required to maintain a constant turnover number for cytochrome c at different oxygen pressures. The PO2 dependence of the turnover of cytochrome c was calculated for many different values for the energy state as a function of the oxygen pressure (see Fig. 4, A and B), whereas the intramitochondrial [NAD+] /[NADH] constant was held at either 0.1 (A) or 10 (B). The energy states and PO2 values for turnover numbers of 1, 3, and 6 s−1 (A) or 3, 6, and 10 s−1 (B) were read for generated data tables and plotted. The graphs show how the energy state changes to maintain the respiratory rate constant as PO2 decreases.
of oxygen pressures, the oxygen-carrying capacity of the saline is <1% that of blood, so the decrease in oxygen pressure in the saline as it flows along the vessels is very rapid. This results in upstream regions of the tissue being well oxygenated, whereas downstream regions are anoxic. Near-infrared absorption measures large volumes of tissue relative to the vascular distribution networks, so the measurements include tissue with the full range of oxygen pressures from well oxygenated to anoxic. The decrease in oxygen pressure in the saline within the vessels and the resultant heterogeneity in myoglobin saturation are interpreted as if it was a homogeneous myoglobin oxygen saturation caused by a radial oxygen gradient in the tissue. The reported large radial oxygen diffusion gradient is misinterpretation of the data. Richardson and colleagues (46–49) presented measurements of myoglobin oxygenation during rest and exercise that were interpreted as evidence that the oxygen pressures in muscle cells are only 2–3 torr during exercise. On first reading the data look quite convincing, but upon further analysis the experimental design has a serious weakness. One part of the data was collected in San Diego, CA, and the other, under matched experimental conditions, in Philadelphia, PA, and the data have been merged and interpreted as if it was a single experiment. Unfortunately, the challenging requirement for doing exercise in the MRI magnet in Philadelphia apparently resulted in restriction of blood flow to the muscle, as evidenced by the reported measurements of phosphocreatine, P_i, and pH. Table II in Richardson et al. (49) shows that during exercise at 64% of maximum in the MRI magnet, the CrP decreased by 83% to only 17% of control, and [P_i] increased more than eightfold. The decrease in CrP reported by others (2, 11, 23, 24, 51, 60) for muscle exercising at 60–100% of maximal work rates ranges from 10 to 40%, with correspondingly smaller increases in [P_i]. Haseler and colleagues (23, 24), for experiments carried out by the same laboratory and at exercise levels matched to those of Richardson et al. (49), reported that phosphocreatine decreased by only 35%. The decrease in intracellular pH in the experiments of Richardson et al. (49) from 7.1 to 6.7 is also much larger than the little or no change reported by other investigators for similar experimental conditions and work rates (2, 11, 23, 24, 51, 60), including the later papers from the same laboratory. The large decreases in pH and CrP reported by Richardson et al. (49) are consistent with the muscle in which myoglobin oxygenation was measured being ischemic. The conclusion by these authors that the intracellular oxygen pressures in muscle cells during normal exercise are 2–3 torr is not supported by the data.

Gayeski and colleagues (15, 16), using microscopic measurements of myoglobin and hemoglobin in rapidly frozen dog Gracilis muscle at near-maximal work rates, reported that red cells in the capillaries indicated PO_2 values of 30–50 torr, whereas the myoglobin was partially desaturated and indicated PO_2 values between 1 and 5 torr. They concluded that there was a large (>20 torr) difference in oxygen pressure between the erythrocytes and the myocytes but that the PO_2 inside myocytes was nearly uniform due to buffering and facilitated diffusion by myoglobin. Although the experiments were done carefully, with many controls, there are several anomalies in the data that indicate that the measured desaturation is not physiological but occurs during freezing of the tissue: 1) Mb was 40–50% desaturated with oxygen even in resting muscles despite the very low rate of oxygen consumption; 2) the fraction of myoglobin with bound oxygen was similar whether the muscles were at rest or working at a rate that increased oxygen consumption by a factor of 50; 3) the measured [CrP]/[Cr] ratio is much lower than that reported for muscle working at 60% of maximum, 0.09 vs. 1–2, indicating extensive breakdown of CrP during freezing; and 4) the large differences in oxygen pressure proposed to occur between the blood plasma and the myocytes in resting muscle are inconsistent with the oxygen pressures in the blood plasma in the microcirculation and the interstitial (pericellular) space of <1 torr measured by phosphorescence quenching (71).

Oxygen diffusion gradients from the extracellular space to the mitochondria. In suspensions of isolated hepatocytes, neuroblastoma, and other small cells, the P50 for oxygen measured by respiratory rate is <1 torr (4, 6, 50, 53). The addition of an uncoupler of oxidative phosphorylation to neuroblastoma cells further decreased the P50 to <0.2 torr (see Ref. 50). For uncoupled mitochondria and cells, the dependence on metabolic parameters other than oxygen is removed. The measured P50 is then the sum of the oxygen dependence of the cytochrome c oxidase reaction and the diffusion gradient between the extracellular space and the mitochondria. If P50 is 0.2 torr, then the oxygen diffusion gradient must be <0.2 torr. The size of the neuroblastoma cells is similar to many of the cells in the body, and they respire with a cytochrome c turnover of ~6 s⁻¹, which is near the average in situ rate (excluding muscle). These small diffusion gradients are consistent with physiological requirements, where the respiratory rates need to be able to increase to cytochrome c turnover numbers of 60–80 s⁻¹ (8, 9, 12, 54, 56, 57) or 10 times the unstimulated rate. Intracellular (extracellular space to the mitochondrial) oxygen diffusion gradients of 0.5 torr at unstimulated rates would increase to 5 torr for a 10-fold increase in rates. This is the upper limit of the diffusion gradient that could exist and still support the maximal turnover numbers for tissues such as brain and liver.

Regulation of cellular energy metabolism. Discussion of the regulation of metabolism, including energy metabolism, often involves the relative contributions of “push” mechanisms such as hormone-induced increases or decreases in the levels of reduced pyridine nucleotides and “pull” mechanisms such as increasing or decreasing metabolic utilization of ATP. Oxidative phosphorylation is coupled to ATP synthesis, and the respiratory rate changes only in response to changes in ATP utilization. When there are large changes in ATP consumption, such as in muscular work, the rate of oxidation of NADH increases in parallel to the rate of oxygen consumption. This requires parallel mechanisms that regulate activity of glycolysis, glycolysis, and the citric acid cycle to match production of NADH to its rate of oxidation. On the other hand, increasing the level of reduction of the NAD pool by hormonal signaling, changing metabolism from sugar to fat, etc., does not affect the rate of respiration unless there is a simultaneous increase or decrease in ATP utilization. As a result, hormonally induced increase in reduction of the NAD pool causes the energy state to increase, whereas a decrease in reduction causes a decrease in energy state. Modulating reduction of the intramitochondrial NAD pool is an important mechanism for regulating the many enzymes and metabolic pathways that are sensitive to the energy state.

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Two experimental observations associated with exercising muscle have attracted considerable attention: 1) the steady-state levels of CrP in muscle increase when the oxygen pressure in the inhaled gas is increased relative to air (see Refs. 23 and 24); and 2) lactate is produced by working muscle under normoxic conditions, and this production is decreased by increasing the oxygen pressure in the inspired gas relative to air (see Refs. 29 and 33). Neither the rate of oxygen consumption nor the work rate changed. Table 1 shows the metabolic response predicted by the model when the PO2 in the inspired gas is changed without the work rate being changed. The capillary PO2 values are only rough estimates, of course, because in vivo there would be a distribution of oxygen pressures, and the oxygen dependence is a nonlinear function of oxygen pressure. For the model, an increase in the cellular PO2 from 13.9 to 58 torr, with a constant cytochrome turnover number of 40 s⁻¹, estimated as 60% of maximum, results in an increase in [CrP] from 28.1 to 108 mM. This is an increase of 8% of the resting concentration. Haseler et al. (24) reported that during submaximal plantar flexion exercise in humans, [CrP] increased by 8–13% when the PO2 in the inspired gas was switched from 0.1 and 1.0 atm. The predicted change is consistent with the experimentally measured change. Since the energy state is coupled to the levels of AMP, the predicted change in energy state would be associated with a change in AMP. Table 1 also shows that when the cellular PO2 is increased from 13.9 to 58 torr, the free [AMP] is predicted to decrease from 1.1 to 0.685 μM, about 60%. Glycogen is the primary carbon source for this type of exercise, and glycogen provides the pyruvate needed for the citric acid cycle to synthesize the NADH for oxidative phosphorylation. AMP is a potent activator of phosphofructokinase, a rate-limiting step in glycolysis. A decrease in AMP concentration by 60% at the same rate of ATP utilization, i.e., no change in pyruvate use by the citric acid cycle, would be expected to decrease lactate production.

Oxygen sensing and control of metabolism. Metabolic oxygen sensors are often enzymes that produce or remove metabolites that contribute to the regulation of metabolism or other physiological activities. Oxidative phosphorylation controls the energy state and thereby the concentration of ADP and, through the activity of adenylate kinase, the concentration of AMP. AMP concentration determines the activity of AMP kinase, which regulates the levels of many enzymes contributing to energy metabolism (7, 20, 39, 52, 61). However, modulation of [AMP] by oxidative phosphorylation is dependent on both PO2 and the rate of ATP utilization; i.e., the effect of decreased oxygen pressure is similar to that for increased ATP utilization, an increase in [AMP]. Sensitivity to PO2 is only part of the sensory function of oxidative phosphorylation, which is to regulate energy metabolism in general. Since oxidative phosphorylation responds to energy metabolism in general and not just to oxygen pressure, it is important for cells to have additional sensors that are more selective for oxygen, such as hypoxia-inducible factor, and have different but often overlapping regulatory functions. However, oxidative phosphorylation can provide accurate and specific response to changing PO2 if the rate of utilization of ATP and the intramitochondrial [NAD⁺]/[NADH] do not change. Under those conditions, the concentrations of ADP, Pi, and AMP are strictly a function of oxygen pressure. This specificity is consistent with, for example, oxidative phosphorylation being responsible for the rapid response of the afferent neural activity of the carotid body to changes in arterial oxygen pressure (72).

In summary, a model of oxidative phosphorylation is presented, for which the steady-state rate expression has been derived and computer programmed. The behavior of oxidative phosphorylation predicted by the model is shown to fit the experimental data available for regulation of oxidative phosphorylation in isolated mitochondria as well as for cells and tissues. Oxidative phosphorylation is programmed to set the metabolic energy state to a precise value and maintain that value with high precision. It is proposed that oxidative phosphorylation, through its central role in setting and maintaining metabolic homeostasis, provided the precise control of metabolism essential to the development of higher plants and animals. Alteration of oxidative phosphorylation through impaired supply of metabolites (oxygen, NADH) or enzyme activity results in pathological disturbance of cellular metabolism and tissue function.

**APPENDIX**

A MatLab program for oxidative phosphorylation was obtained by adding the first two sites of oxidative phosphorylation to the steady-state rate expression for cytochrome c oxidase from Wilson et al. (76):
Ke = 6.4 × 10⁻¹¹;
k₁ = 8 × 10⁻⁹;
k₁r = 8 × 10⁻⁷;
k₂ = 6 × 10⁻⁸;
k₂r = 1 × 10⁻¹;
K₃ = 2 × 10⁻⁶;
K₅ = 1 × 10⁻²⁵;
k₄a = 2.5 × 10⁻⁸;
k₄a = 8 × 10⁻⁷;

a₃t = 1 × 10⁻⁶; % total cytochrome a₃ concentration
ct = 2 × 10⁻⁶; % total cytochrome c concentration (2 × a₃t)
NADt = 2 × 10⁻⁵; % total NAD pool
x = (1 : 10₀)¹; % generates oxygen concentrations

for q = 1 : 16; % generates levels of [CrP]/[Cr][Pi]
W = 7.1; % W = intracellular pH
H = 10⁻⁴ – W; % H = intracellular hydrogen ion concentration
NADH = 1.82 × 10⁻⁵; % sets intramitochondrial NADH
NAD = NADt – NADH; % calculates intramitochondrial NAD+
CrP = 32 – q; % calculates concentration of creatine phosphate (mM)
Cr = 12 + q; % concentration of creatine (mM)
Pi = 3.3 + q; % concentration of Pi (mM)
M = 1. × 10⁻³; * CrP/[Cr] * Pi; % CrP energy state in molar
L = 1.38. × log10(M);
G = 10.36 + L; % calculates free energy CrP hydrolysis
Q = G. / 46.183; % energy state in volts
O = 1. × 10⁻⁸ + x. × 1. × 10⁻⁶ – 6; % O is the oxygen concentration
S = Q/0.059; % coupling value for energy conservation
z = 10⁻⁸S;
k₁f = k₁/z. × 0.5; % couples k₁ to energy state
kr₁ = k₁r. × z. × 0.5; % couples k₁r to energy state
D = (NAD/NADH). × 0.5 × z⁻². × (H. / Ke). × 0.5;
co = D. × ct. / (1 + D); % co is oxidized cytochrome c
ct = ct – co; % ct is reduced cytochrome c
/(k₂. × O); % variable A
B = (k₂. * O. * A + kr₁. × co . * A – k₂r). /
/(k₁f. × cr);
% variable B
C = K₅. × 1. × (1 / H). × (co ./ cr). × 2 × z. × 2 ./ B;
% variable C
III = a₃t. / (1 + K₅. × H + A + B + C); % conc of intermediate III
I = B. × III; % concentration of intermediate I
II = A. × III; % conc of intermediate II
IV = K₃. × H. × III; % conc of intermediate IV
V = C. × III; % conc of intermediate V

y(q,x) = (k₄b. × cr + k₄a. × cr. × k₃. × H) . × III. × 4./ct;
% cyt c TN

end
plot(x,y) % plots cyt c TN vs x value

ACKNOWLEDGMENTS

I thank Mary Leonard for help in preparing the schematic illustrations.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author.

AUTHOR CONTRIBUTIONS

D.F.W. conception and design of research; D.F.W. performed experiments;
D.F.W. analyzed data; D.F.W. interpreted results of experiments; D.F.W.
prepared figures; D.F.W. drafted manuscript; D.F.W. edited and revised
manuscript; D.F.W. approved final version of manuscript.

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