First-Order Kinetics of Muscle Oxygen Consumption, and an Equivalent Proportionality Between Q_{O_2} and Phosphorylcreatine Level

Implications for the Control of Respiration

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ABSTRACT In frog sartorius muscle, after a tetanus at 20°C, during which an impulse-like increase occurs in the rate of ATP hydrolysis, the rate of O_2 consumption (Q_{O_2}) reaches a peak relatively quickly and then declines monoexponentially, with a time constant not dependent on the tetanus duration (τ = 2.6 min in *Rana pipiens* and 2.1 min in *Rana temporaria*). To a good approximation, these kinetics are those of a first-order impulse response, and the scheme of reactions that couple O_2 consumption to extramitochondrial ATP hydrolysis thus behaves as a first-order system. It is first deduced and then demonstrated directly that while $Q_{O_2}(t)$ is monoexponential, it changes in parallel with the levels of creatine and phosphorylcreatine, with proportionality constants $\pm 1/\tau p$, where p is the P/O_2 ratio in vivo. From this, it is further deduced that the mitochondrial creatine kinase (CK) reaction is pseudo–first order in vivo. The relationship between [creatine] and Q_{O_2} predicted by published models of the control of respiration is markedly different from that actually observed. As shown here, the first-order kinetics of Q_{O_2} are consistent with the hypothesis that respiration is rate-limited by the mitochondrial CK reaction; this has as a corollary the "creatine shuttle" hypothesis.

INTRODUCTION

D. K. Hill reported in 1940 that oxygen consumption by a frog muscle after a tetanus at 0°C had a provocatively simple time course. The rate of O_2 consumption (Q_{O_2}) can be calculated as the time derivative of Hill's records. It reached a peak relatively quickly, and its subsequent fall was well fit by a single exponential, whose time constant was essentially the same ($\tau \simeq 9$ min) for all tetani of 2–20 s duration. Hill's method, in which muscle O_2 uptake was measured as an extremely small change in the volume of an O_2-filled chamber, was open to a number of criticisms. However, using an independent method, we recently found (Mahler et al., 1985) the time course of Q_{O_2} in an excised frog muscle after a tetanus at

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20°C to have the same form (albeit four times faster) as that reported by Hill for 0°C (Figs. 2 and 4 below).

Since Hill's work, many aspects of the basic nature of tissue respiration and its control have become apparent. The free energy for all cell function and maintenance appears to be provided by the hydrolysis of ATP, and the ultimate source of almost all ATP produced in muscle is oxidative metabolism. The

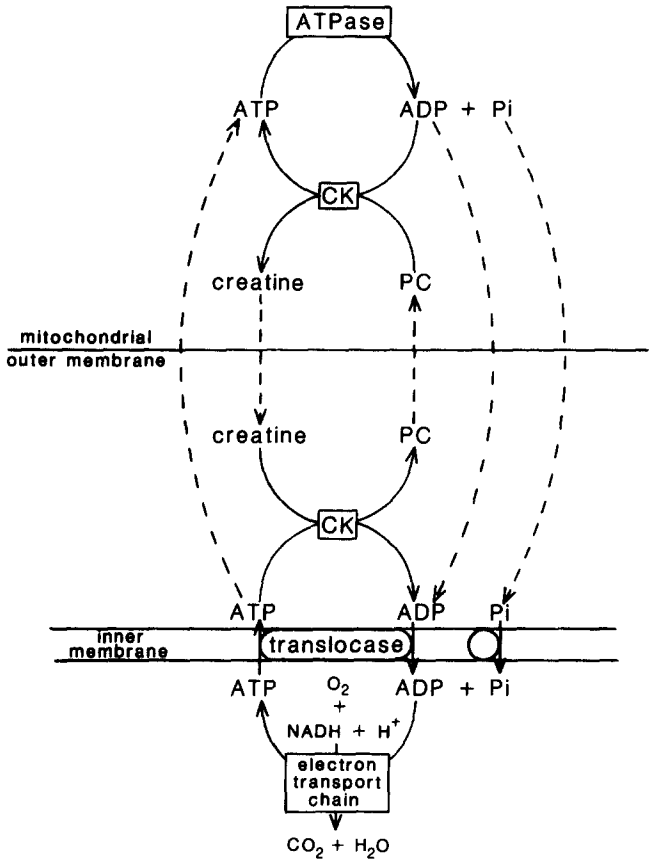

FIGURE 1. Simplified schematic diagram of the reactions by which oxygen consumption is coupled to extramitochondrial ATP hydrolysis in muscle. Dashed lines indicate diffusion.

coupling between these two fundamental processes, whereby a change in the rate of ATP hydrolysis leads to a change in the rate of oxidative phosphorylation, is accomplished by a scheme of reactions whose identity seems well established, but whose quantitative behavior and control mechanisms are still controversial after decades of study (Chance et al., 1962; Owen and Wilson, 1974; Saks et al., 1974, 1978; Davis and Lumeng, 1975; Wilson et al., 1977*b*; Nishiki et al., 1978; Williamson, 1979; Hansford, 1980; Groen et al., 1982; Jacobus et al., 1982). A somewhat simplified, schematic description of these reactions is shown in Fig. 1. A certain fraction of the ADP produced by ATP hydrolysis is rephosphorylated

via creatine kinase (CK); the rest of the ADP can presumably diffuse to the mitochondria. ADP is transported into the mitochondrial matrix, and ATP is transported out, by the adenine nucleotide translocase of the inner membrane, and a carrier also exists for inorganic phosphate (P_i). Within the matrix, the rate of oxidative phosphorylation is some function of the concentrations of ADP, P_i, ATP, O_2, and substrate; the latter term is interpreted broadly to include the redox levels of the components of the electron transport chain. Since CK is also present in the intermembrane space of the mitochondrion, a certain fraction of the ATP leaving the matrix will be used within the mitochondrion to produce phosphorylcreatine (PC); the rest will presumably diffuse to the ATPases.

Attempts to characterize this coupling quantitatively have dealt almost entirely with the properties of its individual components, usually studied in isolated mitochondria. This approach is indispensable, but for a full understanding, it needs to be complemented by observations made on intact muscle. An important test of the validity and relevance of in vitro results, taken as a whole, is that they must account for in vivo behavior. One way to quantify such behavior is to analyze the intact coupling process as a system whose input is the rate of ATP hydrolysis and whose output is the Q_{O_2} (Mahler, 1978). A brief tetanus in a frog muscle at 20°C is accompanied by an impulse-like increase in its rate of ATP hydrolysis (Mahler, 1979; Mahler et al., 1985), and its subsequent time course of Q_{O_2} (Figs. 2 and 4), except for the relatively short delay in reaching a peak, has the same form as the impulse response of a simple first-order system. The coupling process being considered thus behaves as if it were rate-limited by a single step that follows an apparent first-order rate law. Moreover, this behavior is not peculiar to frog muscle; first-order kinetics of Q_{O_2}, while perhaps not quantified as precisely, have been reported for a wide variety of skeletal and cardiac muscles (cf. Discussion). It is surprising that the branching, looping scheme of reactions shown in Fig. 1 should show linear, let alone first-order, behavior, but this appears never to have been addressed in the literature on control of respiration.

A possible clue to the underlying mechanism is the fact that the ATP content of a muscle stays essentially constant, not only during contraction (demonstrated for amphibian [Carlson, 1963; Mommaerts, 1969; Gilbert et al., 1971; Homsher et al., 1975; Dawson et al., 1977], avian [Arese et al., 1965], and mammalian muscle [Edwards et al., 1972; Gower and Kretzschmar, 1976; Crow and Kushmerick, 1982]), but also during recovery (Dawson et al., 1977; Arese et al., 1965; Edwards et al., 1972; Piiper and Spiller, 1970; Kushmerick and Paul, 1976). Given this fact, it can be deduced that when $Q_{O_2}(t)$ is monoexponential, it must be changing in parallel with the tissue contents of PC and creatine, as stated in Eq. 1:

$$\Delta Q_{O_2}(t) \simeq \frac{-1}{\tau p}\,\Delta\{\mathrm{PC}\}(t) = \frac{1}{\tau p}\,\Delta\{\mathrm{creatine}\}(t). \tag{1}$$

Here {PC} and {creatine} denote the levels[1] of PC and creatine; $\Delta Q_{O_2}(t)$, $\Delta\{\mathrm{PC}\}(t)$,

[1] The terminology and notation of Hohorst et al. (1962) are used, in which the tissue level of substance A, denoted $\{A\}$, designates the total content of A per unit weight. The symbol $[A]$ is reserved for the actual concentration of A known to be free in solution within the tissue or relevant compartment.

and $\Delta\{\text{creatine}\}(t)$ are the changes in Q_{O_2}, {PC}, and {creatine} from their basal values, at time t, τ is the time constant of the Q_{O_2}, and p is the P/O_2 ratio for oxidative metabolism, which has an expected value of ~6.3 mol/mol (Mahler, 1979; Crow and Kushmerick, 1982). A formal derivation of Eq. 1 is given in the Appendix, but its essence is the following. The absence of measurable change in {ATP} during recovery implies that the production of ATP by oxidative metabolism must be followed almost instantaneously by its virtually stoichiometric dephosphorylation via CK. Presumably, this occurs within the mitochondrion (Jacobus and Lehninger, 1973; Fig. 1), and PC can thus be thought of as the end product of oxidative phosphorylation. If suprabasal oxidative metabolism is phosphorylating creatine with a monoexponential time course, it must be doing so at a rate proportional to $\Delta\{\text{creatine}\}$. The first goal of the work described in this paper was to provide a rigorous test of this prediction in the frog sartorius muscle.

For a wide range of changes in ΔQ_{O_2} and $\Delta\{PC\}$, the relationship between them was in fact well described by the proportionality predicted in Eq. 1. This relationship was then used to test a widely accepted model for control of respiration in muscle, first proposed by Chance et al. (1962), which postulates (see Fig. 1) continuous near-equilibrium of CK and a first-order Michaelis-Menten dependence of Q_{O_2} on cytoplasmic [ADP], in a well-stirred cytoplasm. Finally, because the relationship between Δ[PC] and ΔQ_{O_2} predicted by this model differed drastically from the one actually observed, the latter was used to formulate the alternative hypothesis that respiration is rate-limited by the mitochondrial CK reaction; this has as a corollary the controversial "creatine shuttle" hypothesis (Jacobus and Ingwall, 1980; Brautbar and Bessman, 1985).

METHODS

$\Delta Q_{O_2}(t)$ was measured by the method described in the preceding paper (Mahler et al., 1985), according to the computational algorithm

$$\Delta Q_{O_2}(t) \simeq \mathcal{F}_D^{-1}\left\{\frac{\mathcal{F}_D[\Delta P_{O_2}(t)](\omega)}{H(j\omega)}\right\}(t). \tag{2}$$

All measurements were made at 20°C. For tetani of 3.2–10 s, $P_{O_2}(t)$ at the muscle surface was sampled every 6 s. If the P_{O_2} fell to zero, $\Delta Q_{O_2}(t)$ could still be calculated for times up to this point. With Eq. 2, when $P_{O_2}(t)$ does not return to approximately to its initial value, large computational errors occur (Brigham, 1974), and this was certainly the case when $P_{O_2}(t)$ reached zero ($\Delta P_{O_2} \simeq -500$ mmHg; Fig. 3). To prevent such errors, an artificial time course was appended to the measured $P_{O_2}(t)$, which gradually led it back to its starting value. Measured values of <20 mmHg were discarded to ensure that $Q_{O_2}(t)$ remained independent of $P_{O_2}(t)$ (D. K. Hill, 1948). The appended time course was held constant for 5–20 points at the lowest acceptable measured value of P_{O_2}, and then increased linearly to reach the starting value after another 10–30 points. The number of points in the entire time course was usually 64, but was occasionally 32. To determine the effect of this procedure on the calculated $\Delta Q_{O_2}(t)$, it was used in the case of a shorter tetanus, after which $P_{O_2}(t)$ had returned to its starting value. The P_{O_2} record was interrupted at a certain point and an artificial time course, constructed as just described, was substituted for the remaining measured values. $\Delta Q_{O_2}(t)$ was calculated from this hybrid record and, for the times prior to the interruption, was found to differ negligibly from the values calculated

from the full P_{O_2} record, except at the one or two points just before the interruption, at which larger-than-normal oscillations were evident. Accordingly, the final two values were discarded from each time course of ΔQ_{O_2} calculated from an incomplete record of $P_{O_2}(t)$.

For the measurement of changes in the levels of muscle metabolites during contraction and recovery, rapid freezing of pairs of muscles was done by the method of Homsher et al. (1975). All experiments were done at 20°C, and one muscle of each pair was tetanized isometrically with just-supramaximal stimuli of 0.6 ms duration and 70/s frequency. The contralateral muscle served as a control. The preparation of muscle extracts, and the measurement of their contents of creatine and PC and, in some cases, ATP and ADP, was done by the methods of Homsher et al. (1972). In later experiments, the determination of the contents of ATP, ADP, and AMP in the extracts was done using high-pressure liquid chromatography, as described by Dubyak and Scarpa (1983).

For the measurement of the time course of change in the levels of tissue metabolites after a tetanus of 0.5 s in the sartorius of *Rana temporaria*, groups of paired muscles were frozen at 0.4, 60, 110, 150, and 240 s and 20 min after the cessation of stimulation. For the measurement of the levels of tissue metabolites at time t_{peak} after a tetanus, where t_{peak} was defined as the time at which $\Delta Q_{O_2}(t)$ reached its maximum, t_{peak} was determined from the mean response of $\Delta Q_{O_2}(t)$, which was based on at least nine experiments for each tetanus duration. For tetani of 0.2–1.0 s, $\Delta Q_{O_2}(t)$ was measured at 24-s intervals, and for tetani of 3.2–10 s, it was measured at 6-s intervals. For tetani of 0.2, 0.5, 1, 3.2, 5, and 10 s, respectively, t_{peak} was found to be 96, 72, 48, 36, 24, and 24 s after the beginning of stimulation. For tetani of 3.2–10 s, groups of paired muscles were frozen at the appropriate t_{peak}. For tetani of 0.2–1.0 s, $\Delta\{PC\}(t_{peak})$ was calculated from the values of $\Delta\{PC\}_0$ reported by Mahler (1979), measured 0.4 s after the end of stimulation. Two corrections were made to these values. The first was the amount of PC produced via oxidative metabolism between the time of measurement and t_{peak}, calculated as $p \cdot \int_0^{t_{peak}} \Delta Q_{O_2}(t)dt$, where p is the P/O_2 ratio for oxidative metabolism, which was assumed to be 6.3 mol/mol. The second was the amount of postcontractile suprabasal PC utilization (see Mahler et al., 1985), which was assumed to be 0.24 μmol/g, the cost of calcium pumping calculated from the data of Somlyo et al. (1982). If the latter correction were omitted or doubled, it would not affect the conclusions drawn from this set of experiments (see Fig. 5 below).

To check for the existence of mitochondrial CK in the frog sartorius, a modification of the method of Hall and DeLuca (1976) was used. Muscles were frozen, powdered, and suspended in 10 mM Tris-HCl, pH 7.2, supplemented with 1 mM dithiothreitol. To solubilize mitochondrial CK, the detergent NP-40 was added to a final concentration of 1%, and the suspension was incubated for 30 min. Thereafter, the procedure of Hall and DeLuca (1976) was followed. The existence of the mitochondrial isozyme of CK was confirmed by the presence, after electrophoresis and fluorescence scanning, of a small peak closer to the cathode than that of the MM isozyme (Hall and DeLuca, 1976; Hall et al., 1977), which accounted for <1% of the total CK activity of the muscle (~4,000 IU/g at 23°C, pH 6.6).

RESULTS

Tests of Eq. 1

The proportionality between $\Delta Q_{O_2}(t)$ and $\Delta\{PC\}(t)$ predicted by Eq. 1 was tested by measuring both variables under identical or equivalent conditions, according to two protocols. In the first, measurements were made after a tetanus of 0.5 s in the sartorius of *R. temporaria* (Fig. 2, *A* and *B*). Using $\tau = 2.12$ min (Fig. 2*A*)

and p = 6.3 μmol/μmol, the predicted proportionality constant, $-1/\tau p$, was -0.0748 μmol O_2/min·μmol PC. After normalization by total creatine (C_T) content (Carlson et al., 1967), the observed line of best fit relating $\Delta Q_{O_2}(t)$ to $\Delta\{PC\}(t)$ had an intercept not significantly different from zero [$(-1.19 \pm 2.42) \times 10^{-4}$], a correlation coefficient of -0.993, and a slope of -0.0777 ± 0.0055. If the line was constrained to pass through the origin, its slope was -0.0755. No significant changes were observed in {ATP} or {ADP}.

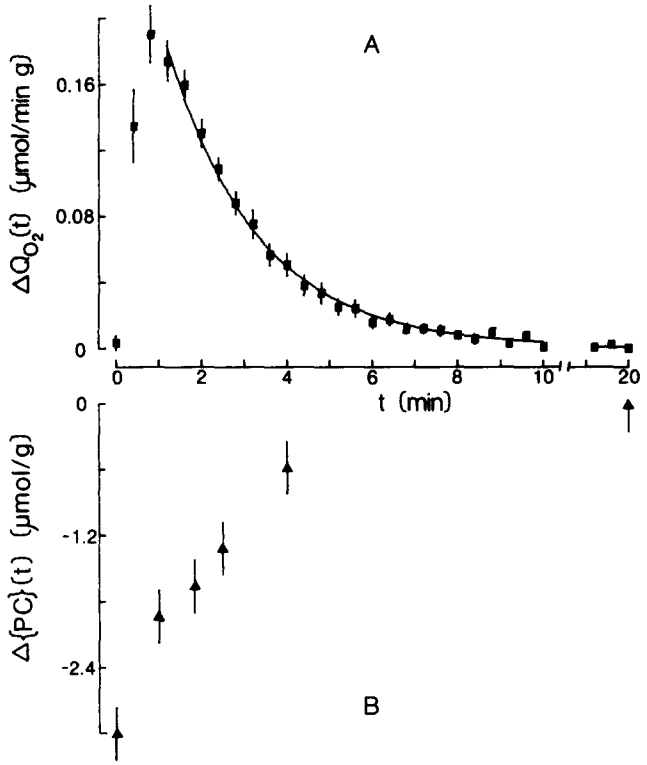

FIGURE 2. Simultaneous time courses of ΔQ_{O_2} (*A*) and $\Delta\{PC\}$ (*B*) in the sartorius of *R. temporaria* after an isometric tetanus of 0.5 s at 20°C. Error bars indicate ± SE. The continuous curve in *A* is the best-fitting monoexponential (τ = 2.12 min).

Despite the good overall agreement between prediction and observation, this test of Eq. 1 was handicapped by the relatively large variability in the values of $\Delta\{PC\}$. For example, $\Delta\{PC\}(t)$ (Fig. 2*B*) was not very well approximated by its best-fitting exponential (τ = 2.2 min), but there were no statistically significant differences between the measured values and those of best fit. This variability was probably due largely to the fact that measured changes in $\{PC\}/\{C_T\}$, the largest of which was -0.0655 ± 0.0044 (n = 11), were rather small relative to its resting value, 0.793 ± 0.004 (n = 52).

In order to test Eq. 1 over a wider range of values of $\Delta\{PC\}$ and ΔQ_{O_2}, a second protocol was devised. Sartorii of *R. pipiens* were tetanized isometrically for up to

10 s. As with shorter tetani, $\Delta Q_{O_2}(t)$ could be determined by measuring the time course of P_{O_2} at the closed, lower surface of the muscle while the P_{O_2} at its upper surface was held constant, and numerically solving the diffusion equation for $\Delta Q_{O_2}(t)$. For tetani of 5 s or longer, the P_{O_2} at the lower surface of the muscle usually fell to zero within 2–3 min, even when the P_{O_2} at the upper surface exceeded 700 mmHg (Fig. 3*A*). With the proper mathematical precautions,

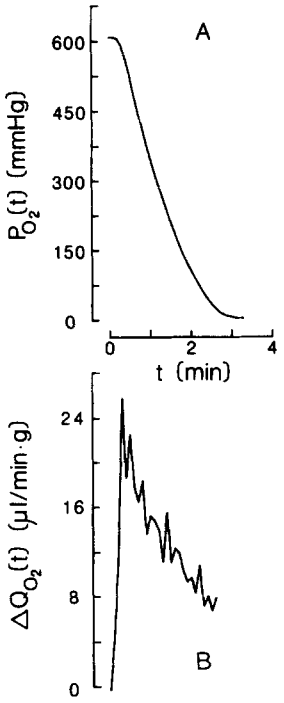

FIGURE 3. (*A*) Typical time course of change in P_{O_2} at the center of the closed, lower surface of the sartorius of *R. pipiens* during and after an isometric tetanus of 10 s at 20°C, begun at $t = 0$. The P_{O_2} at the upper surface was kept constant at 701 mmHg. (*B*) Time course of ΔQ_{O_2} calculated from the record in *A* by the diffusion method.

however, it was possible to calculate $\Delta Q_{O_2}(t)$ for the period during which the muscle was oxygenated, which was always long enough to allow Q_{O_2} to reach its peak (Fig. 3*B*). Moreover, in some cases it was possible to obtain a relatively complete time course of $\Delta Q_{O_2}(t)$ after a tetanus of 5 or 10 s, and its form was practically identical to that after a tetanus of 0.1–1.0 s (Fig. 4). Its peak was earlier and sharper, but in most cases the descending limb was again adequately described by a single exponential whose time constant averaged ~2.5 min. Previously, it was observed that for tetani of 0.2, 0.5, and 1.0 s in this muscle, the values of τ that best fit the mean time course of Q_{O_2} were 2.75 (Fig. 4*A*), 2.76, and 2.46 min, respectively (Mahler et al., 1985), and for the 35 individual records on which those mean responses were based, the rate constant $1/\tau$

averaged 0.380 ± 0.019 min^{-1}, which corresponds to a value of 2.63 min for τ. These results indicate that the proportionality between $\Delta\{PC\}$ and ΔQ_{O_2} predicted by Eq. 1, which was based on the monoexponential kinetics of $\Delta Q_{O_2}(t)$, should also be valid during the descending phase of ΔQ_{O_2} after a tetanus of up to 10 s.

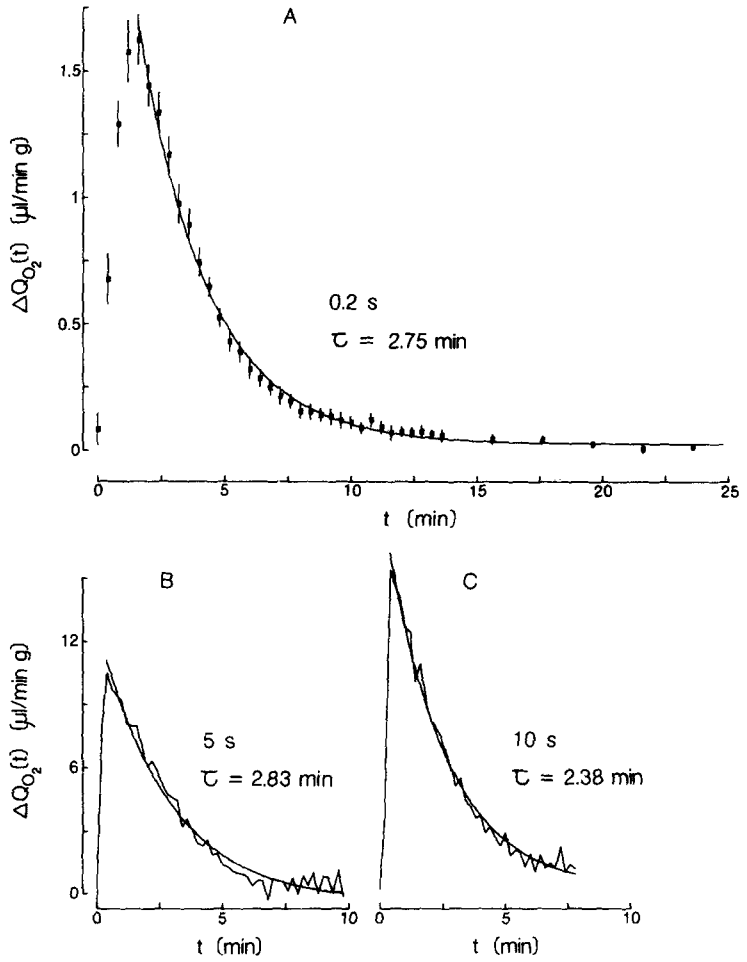

FIGURE 4. (*A*) Mean time course of $\dot{Q}_{O_2}$ in the sartorius of *R. pipiens* after a tetanus of 0.2 s at 20°C, with the best-fitting monoexponential through the descending limb. Error bars designate ± SE. (*B* and *C*) Individual records of the time course of ΔQ_{O_2} in the sartorius of *R. pipiens* after tetani of 5 and 10 s at 20°C, with best-fitting monoexponentials.

A 10-s tetanus presented a severe challenge to the contractile and metabolic capability of the muscle. Tension fell to 42 ± 2% (n = 17) of its initial maximum. $\Delta Q_{O_2}(t)$, measured at 6-s intervals, had a peak value, reached 24 s after the beginning of stimulation, of 0.831 ± 0.047 (n = 8) μmol O_2/min·g, which is nearly 30 times the resting $\dot{Q}_{O_2}$ (0.0294 ± 0.0015 [n = 11] μmol/min·g). Given τ = 2.6 min and p = 6.3 μmol/μmol, Eq. 1 predicted that $\{PC\}(t)$ would

simultaneously have dropped to about half its resting value. Accordingly, rather than tracking $\Delta\{PC\}$ and ΔQ_{O_2} during a single metabolic episode, I decided to compare them at the times when ΔQ_{O_2} after a tetanus was at its peak, for a range of tetanus durations up to 10 s. These times were denoted t_{peak}.

For tetani of 3.2–10 s, the values of $\Delta Q_{O_2}(t_{peak})$ were particularly trustworthy, since they were virtually independent of the value used for the diffusion coefficient for O_2 (D) or of the configuration used to approximate the tissue boundary. In general, the time course of P_{O_2} measured at the center of the lower surface of the muscle is determined by two processes: the consumption of O_2 and its diffusion to that point. However, during the first 30 s or so after a tetanus, the rate at which O_2 diffuses to this point is negligible in comparison with the rate at which it is being consumed. The proof of this is that for these times, the calculated $\Delta Q_{O_2}(t)$ is essentially unaffected by a fivefold change in D in either direction, or by describing the tissue as a sheet or a hemisphere rather than a hemi-elliptical cylinder. Thus, as is evident from Fig. 3, to an excellent approximation the rate of change in the measured P_{O_2} is simply proportional to $\Delta Q_{O_2}(t)$, with the proportionality constant being $1/\alpha$, where α denotes the solubility of O_2 in the tissue. Conversely, for a precise determination of $\Delta Q_{O_2}(t)$ from $\Delta P_{O_2}(t)$ at these early times, one needs only an accurate value for α, which is given in the preceding paper (Mahler et al., 1985). It was unmistakable that ΔQ_{O_2} and $\Delta\{PC\}$ closely mirrored each other (Fig. 5), and it was again impossible to distinguish statistically between the measured values and those predicted by Eq. 1. The predicted proportionality constant was -0.0611 μmol O_2/min·μmol PC; after normalization by $\{C_T\}$, the best-fitting line relating $\Delta Q_{O_2}(t_{peak})$ to $\Delta\{PC\}(t_{peak})$ had an intercept of $(-1.07 \pm 1.12) \times 10^{-3}$, a slope of -0.0682 ± 0.0054, and a correction coefficient of -0.988. If the line was constrained to pass through the origin, the best-fitting slope was -0.0637. $\Delta\{ATP\}$ and $\Delta\{ADP\}$ were rather variable, but were never significantly different from zero (Table I).

Because the coupling of the reverse CK reaction to the hydrolysis of ATP and that of the forward CK reaction to the oxidative production of ATP are evidently both practically stoichiometric (see Discussion), the aggregate of all these reactions can, to an excellent approximation, be represented simply by

$$PC \rightleftharpoons \text{creatine} + P_i. \tag{3}$$

Normally, the net production and consumption of P_i by other reactions is negligible in comparison, and $\Delta\{P_i\}$ closely matches $\Delta\{\text{creatine}\}$ (Homsher et al., 1975; Kushmerick and Paul, 1976). Thus, the relationship

$$\Delta Q_{O_2}(t) \simeq \frac{1}{\tau p}\,\Delta\{P_i\} \tag{4}$$

should also be valid while $\Delta Q_{O_2}(t)$ is monoexponential.

Use of Eq. 1 to Test Models for Control of Respiration

In 1962, Chance et al. proposed a model for the control of respiration in muscle, which postulated (*a*) that CK is continuously near equilibrium, and (*b*) that the Q_{O_2} is determined by cytoplasmic [ADP], according to a first-order Michaelis-Menten relationship. In addition, it was tacitly assumed (*c*) that the cytoplasm is

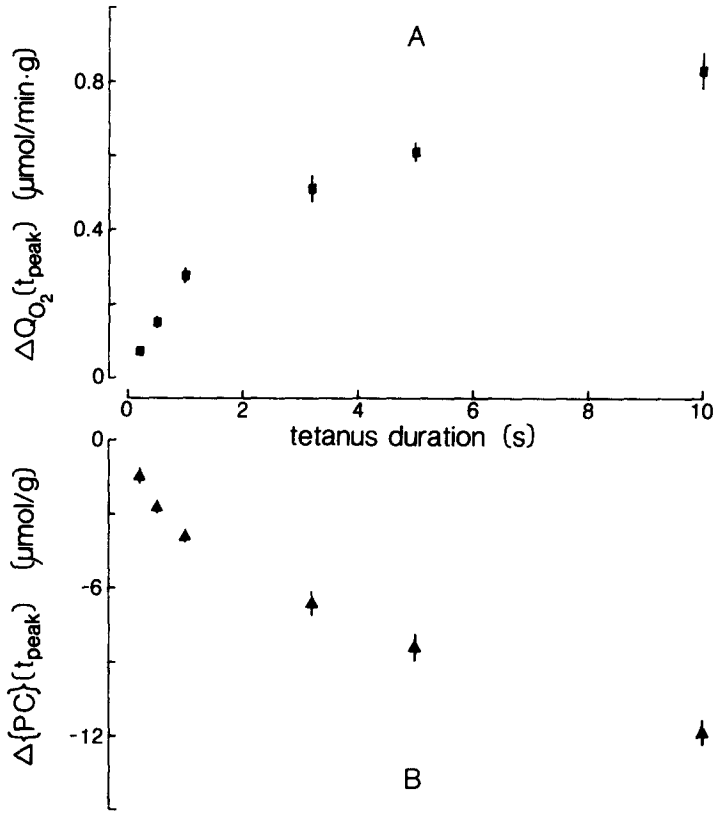

FIGURE 5. (*A*) Peak values of ΔQ_{O_2} in the sartorius of *R. pipiens* after tetani of 0.2–10 s at 20°C, plotted as a function of the tetanus duration. (*B*) Simultaneous values of Δ{PC}. Error bars designate ± SE.

well stirred, and (*d*) that [ATP] + [ADP] stays constant. Since then, a number of important aspects of the control of respiration have become apparent, but the assumptions of this model remain plausible (see Discussion). However, as also discussed below, the in vivo behavior used to test the model has been quite limited, and its success in accounting for even this behavior is questionable. Because the pseudo-first-order kinetics of ΔQ_{O_2} and the equivalent proportion-

TABLE I

Changes in Levels of ATP and ADP in the Sartorius of R. pipiens after Isometric Tetani of 3.2–10 s at 20°C

	Tetanus duration		
	3.2 s	5 s	10 s
Δ{ATP}/{C_T} (nmol/μmol)	1.4±1.3	0.6±0.77	1.4±1.6
	(18)	(8)	(9)
Δ{ADP}/{C_T} (nmol/μmol)	0.14±0.29	0.46±0.22	0.57±0.50
	(18)	(8)	(9)

Values are means ± SE. Numbers in parentheses are degrees of freedom.

ality between $\Delta\{PC\}$ or $\Delta\{creatine\}$ and ΔQ_{O_2} are certainly among the most distinctive properties of respiration in intact muscle, it was of considerable interest to determine whether they could be accounted for by this model. It is a straightforward matter to show that the relationship between $\Delta\{creatine\}$ and ΔQ_{O_2} predicted by the model, which is valid for both steady and non-steady states, has the form

$$\Delta Q_{O_2} = \frac{C_1 \Delta\{creatine\}}{C_2 - \Delta\{creatine\}}, \tag{5}$$

where C_1 and C_2 are positive constants. Eq. 5 can be deduced as follows. Let the concentrations of MgATP, MgADP, PC, and creatine be denoted T, D, P, and C, and let Q_{O_2} be denoted Q; let the subscript 0 denote the basal value, and let the prefix Δ denote the change from the basal value. The model postulates that

$$DP/TC \simeq K_{CK}/[H^+], \tag{6.1}$$

$$Q = Q_{max} D/(K_M + D), \tag{6.2}$$

$$T + D = T_0 + D_0. \tag{6.3}$$

In Eq. 6.1, K_{CK} denotes the equilibrium constant for CK. We will denote $K_{CK}/[H^+]$ by K^*, and assume for convenience that $[H^+]$, and thus K^*, stay constant (Dawson et al., 1977; Meyer et al., 1982). However, it appears that pH changes within a range of even ± 0.5 would not materially affect the conclusions drawn from this analysis. To express ΔQ as a function of ΔC, Eq. 6.2 is used to write ΔQ as a function of ΔD, and Eqs. 6.1 and 6.3 are used to write ΔD as a function of ΔC. First note that Eq. 6.2 implies that

$$\Delta Q = \Delta Q_{max} \cdot \Delta D/[(K_M + D_0) + \Delta D]. \tag{7.1}$$[2]

Eq. 6.1 can be rewritten as

$$K^* \simeq (D_0 + \Delta D)(P_0 + \Delta P)/(T_0 + \Delta T)(C_0 + \Delta C). \tag{7.2}$$

From Eq. 6.3, $\Delta T = -\Delta D$; similarly, $\Delta P = -\Delta C$. Substituting these relationships into Eq. 7.2 yields

$$K^* \simeq (D_0 + \Delta D)(P_0 - \Delta C)/(T_0 - \Delta D)(C_0 + \Delta C), \tag{7.3}$$

[2] To see this, note that

$$Q_0 = Q_{max}\, D_0/(K_M + D_0), \tag{7.11}$$

so that

$$\begin{aligned} \Delta Q = Q - Q_0 &= Q_{max}\left(\frac{D}{K_M + D} - \frac{D_0}{K_M + D_0}\right) \\ &= Q_{max}\frac{K_M}{K_M + D_0}\frac{\Delta D}{K_M + D}. \end{aligned} \tag{7.12}$$

But, from Eq. 7.11,

$$\Delta Q_{max} = Q_{max} - Q_0 = Q_{max}\, K_M/(K_M + D_0), \tag{7.13}$$

and substituting this into Eq. 7.12 yields Eq. 7.1.

and by algebraic manipulations it follows that

$$\Delta D \simeq \frac{(D_0 + K^*T_0)\Delta C}{(P_0 + K^*C_0) - (1 - K^*)\Delta C}. \tag{7.4}$$

Substituting Eq. 7.4 into Eq. 7.1 yields Eq. 5, with

$$C_1 = \frac{\Delta Q_{max}(D_0 + K^*T_0)}{K_M(1 - K^*) - K^*(D_0 + T_0)}, \tag{8.1}$$

$$C_2 = \frac{(K_M + D_0)(P_0 + K^*C_0)}{K_M(1 - K^*) - K^*(D_0 + T_0)}. \tag{8.2}$$

As long as Δ[creatine] is much smaller than C_2, the predicted relationship between Δ[creatine] and ΔQ_{O_2} will approximate a proportionality, with slope $C_1/$

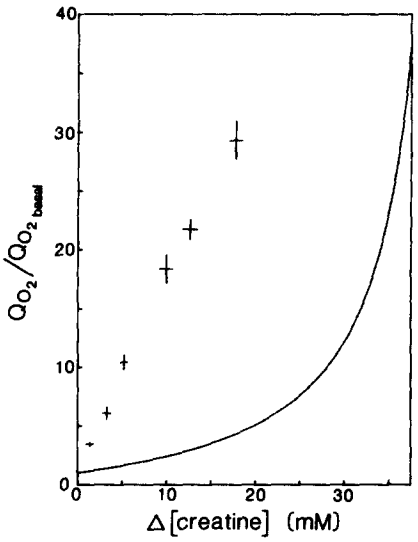

FIGURE 6. Relationships between Δ[creatine] and Q_{O_2}/(basal Q_{O_2}). The smooth curve is the relationship predicted by the model of Chance et al. (1962), with parameter values chosen to simulate the frog sartorius muscle at 20°C, as described in the text. The data are those of Fig. 5, with the assumption that $[X] = 1.5\ \{X\}$ (see text).

C_2. However, it appears that for any plausible set of parameter values, Eq. 5 must be markedly nonlinear over the physiological range of Δ[creatine]. For example, as shown in Fig. 6, for parameter values chosen to simulate the frog sartorius at 20°C, the predicted relationship differs drastically from that actually observed. For this case, the basal values of [ATP], [PC], and [creatine] were 7.5, 37.5, and 12 mM, respectively. These were calculated from typical values of {ATP}, {PC}, and {creatine} (5, 25, and 8 μmol/g, respectively), and from the assumptions that free intracellular water accounts for two-thirds of muscle weight and that these metabolites are not bound or compartmentalized, so that [X] (mM) = 1.5 {X} (μmol/g). K^* was assumed to be 1/166 at pH 7 (Lawson and Veech,

1979), and model assumption *a* (Eq. 6.1) forced the basal [ADP] to be 14.5 μM. From assumption *b* (Eqs. 6.2 and 7.11), it follows[2] that

$$K_M/D_0 = (Q_{max}/Q_0) - 1. \tag{7.14}$$

As discussed above, measurements showed that Q_{max}/Q_0 was at least 30, and it was assumed to be 40; this set K_M at 0.564 mM. The accuracy of some of these parameter values, particularly K_M and, to a lesser extent, D_0, K^*, and Q_{max}, can be questioned. More generally, it is natural to ask whether any plausible sets of parameter values can be found for which the predicted curve is consistent with observation. With regard to the first question, it should be kept in mind that only certain combinations of parameter values are allowed by the model. For example, if a lower value of K_M is desired, then according to Eq. 7.14, D_0 or Q_{max}/Q_0 must also be lowered. However, the latter can be decreased no more than ~25% without contradicting the direct observations described above, so if K_M is to be greatly decreased, an approximately proportional change must be made in D_0. It is certainly possible that the concentration of ADP in resting muscle is substantially less than 14.5 μM; it can currently be directly measured only with ^{31}P nuclear magnetic resonance (NMR), and its value is smaller than the present measurement error of this method (Meyer et al., 1982; Matthews et al., 1982; Dubyak and Scarpa, 1983). However, since the values used here for T_0, P_0, and C_0, the resting concentrations of ATP, PC, and creatine, seem likely to be quite accurate, then according to the assumption of near-equilibrium of C_K (Eq. 6.1), D_0 can be decreased substantially only if K^* is decreased proportionately, to a value much lower than that estimated by Lawson and Veech (1979).

Postponing concerns about the compatibility of the model assumptions, it appears that the second question posed above can be answered definitively. A closer look at Eqs. 8.1 and 8.2 shows that there is no plausible set of parameter values permitted by the model for which the predicted relationship between Δ[creatine] and ΔQ_{O_2} can adequately fit the data shown in Figs. 2 and 5; the discrepancy between prediction and observation must always be similar to that shown in Fig. 6. The basis for these conclusions is, first, that the constant C_2 appearing in Eq. 5 can never be much larger than the resting concentration of PC, which sets the upper limit for Δ[creatine]. This, if Δ[creatine] changes through any appreciable fraction of its physiological range, the condition Δ[creatine] $\ll C_2$, which is necessary for linearity of Eq. 5, will not hold. In addition, it can be shown that C_1/C_2, the slope of the linear portion of Eq. 5, must always be substantially less than that established by the data or by Eq. 1.

The easiest way to make these deductions is by substituting into Eqs. 5.1 and 5.2 approximations that will be consistent with any plausible set of parameter values. These are

$$D_0 \ll K_M, \tag{9.1}$$

$$K^* \ll 1, \tag{9.2}$$

$$K^*C_0 \ll P_0, \tag{9.3}$$

$$D_0 \ll T_0. \tag{9.4}$$

Eq. 9.1 follows from Eq. 7.14 and any plausible value of Q_{max}/Q_0. Eq. 9.2 will hold for any plausible value of K^*, and Eq. 9.3 follows from Eq. 9.2 and any plausible values of C_0 and P_0. Eq. 9.4 follows directly from Eq. 9.3 when K^*C_0 is written as D_0P_0/T_0. Substituting these inequalities into Eq. 8.2 yields

$$C_2 \simeq \frac{K_M P_0}{K_M - K^* T_0}. \tag{10.1}$$

The term $K^*T_0 = D_0P_0/C_0$, and by Eq. 7.14, $D_0 = K_M/(\Delta Q_{max}/Q_0)$. Making these substitutions in Eq. 10.1 allows the terms K_M to be cancelled, and leaves

$$C_2 \simeq P_0 \left[\frac{(\Delta Q_{max}/Q_0)}{(\Delta Q_{max}/Q_0) - (P_0/C_0)} \right]. \tag{10.2}$$

For the parameter values chosen above, the term in parentheses is 1.09, and it appears that for any plausible parameter values, it can never be much greater than 1. An exact evaluation of Eq. 8.2 for the case at hand gives $C_2 = 1.12\ P_0$, which verifies that this approximation is substantially accurate.

In a similar fashion, using the exact expression furnished by Eqs. 8.1 and 8.2 for C_1/C_2, the slope of the linear portion of the predicted relationship between Δ[creatine] and ΔQ_{O_2} (Eq. 5), together with the approximations 9.1 and 9.3, it follows that

$$C_1/C_2 \simeq Q_0(P_0 + C_0)/P_0C_0. \tag{11}$$

Q_0, the basal Q_{O_2}, can confidently be set at ~33 nmol O_2/min·g (Mahler et al., 1985, Table III). Using the values chosen above for P_0 and C_0, the resting concentrations of PC and creatine, Eq. 11 predicts that $C_1/C_2 \simeq 5.4$ nmol O_2/min·μmol creatine. This is <10% of the observed proportionality constant (Fig. 6), which was 64 nmol O_2/min·μmol creatine. Moreover, Eq. 11 shows that a similar discrepancy between the predicted and observed slopes must exist for any plausible set of parameter values. Eqs. 10.2 and 11 emphasize that the relationship between Δ[creatine] and ΔQ_{O_2} predicted by the model is primarily determined by the relatively well-established values of the parameters Q_0, Q_{max}/Q_0, P_0, and C_0, and is little influenced by the values used for T_0, D_0, K^*, and K_M, as long as the conditions stated in Eqs. 9.2 and 9.4 are satisfied. This is not surprising when one considers that, because of the relationships among the parameters established by the model assumptions (Eqs. 6.1 and 6.2), once any four of K^*, T_0, D_0, P_0, and C_0 and either of K_M or Q_{max}/Q_0 are chosen, the remaining two values are also fixed.

DISCUSSION

General Validity of Eq. 1

The pseudo–first-order kinetics of Q_{O_2} reported here for excised frog muscle have been more or less apparent in previous studies with this preparation (D. K. Hill, 1940*a*; Kushmerick and Paul, 1976). In those experiments, O_2 uptake by the muscle, rather than O_2 consumption per se, was measured. The difference between the two is the change in the O_2 content of the tissue, which can be

calculated via the diffusion equation for O_2, and is relatively large at certain times (Mahler et al., 1985). The application of this correction by previous workers, while approximate, was evidently substantially correct and revealed the general form of the time course of the Q_{O_2} after a tetanus (D. K. Hill, 1940*a*; Kushmerick and Paul, 1976) and its independence of tetanus duration over a wide range (Hill, 1940*a*).

It is also apparent from the literature that the pseudo–first-order kinetics of Q_{O_2} are not peculiar to frog muscle. Piiper et al. (1968) reported that in the in situ dog gastrocnemius during a series of brief contractions, which simulated a step increase in the rate of ATP hydrolysis, the rate of O_2 uptake rose to steady state with a time course that could be approximated by a single exponential, whose time constant (τ = 24 s) did not depend on the steady state Q_{O_2}. In this case, as with all preparations in which O_2 is supplied via the circulation, the rate of O_2 uptake can be expected to provide a good approximation to the Q_{O_2}, since the diffusion distances for O_2 are short enough that changes in the O_2 store of the muscle are small relative to the amounts consumed. Crow and Kushmerick (1982) reported that after a single tetanus by an excised mouse soleus or extensor digitorum longus (EDL) at 20°C, the time course of its rate of O_2 uptake from a closed chamber was well described by a first-order model, with τ = 0.6 min for the soleus and 2.3 min for the EDL. These measurements were not corrected for the effect of the O_2-measuring system (τ = 0.25 min) or for the diffusional delay between O_2 consumption and uptake. However, because of the small size of the muscles (≃5 mg), the latter correction can be expected to be small relative to that for the excised frog sartorius (see Fig. 8 of Mahler et al., 1985), and the results of Crow and Kushmerick imply that the time course of Q_{O_2} must also have been well approximated by a first-order response. The time course of dihydronicotinamide adenine dinucleotide (NADH) fluorescence in an excised rabbit papillary muscle after a twitch or a series of twitches at 23°C has a form virtually identical to that reported here for the time course of Q_{O_2} in the frog sartorius after a tetanus (Figs. 2 and 4), albeit 20–25 times faster, with τ = 6.7 s (Chapman, 1972). It has been demonstrated in both isolated heart mitochondria (Chance et al., 1962) and intact skeletal muscle that under conditions of adequate oxygenation, the time course of change in NADH fluorescence gives an excellent approximation to the time course of ΔQ_{O_2}. For intact muscle, this follows from separate comparisons of the time course of the rate of recovery heat production to those of NADH fluorescence (Godfraind-De Becker, 1972) and Q_{O_2} (Hill, 1940*a*, *b*; Mahler et al., 1985). Taken together, these results imply that the Q_{O_2} also exhibits pseudo–first-order kinetics in rabbit papillary muscle, and it is natural to speculate that this may be a general property of all vertebrate skeletal and cardiac muscle.

Because the proportionality between Δ[PC] or Δ[creatine] and ΔQ_{O_2} stated in Eq. 1 follows directly from the pseudo–first-order kinetics of ΔQ_{O_2} and the constancy of [ATP], it should similarly be a widespread property of skeletal and cardiac muscle. Fig. 7 illustrates a convenient test of this prediction, using the present data together with those from what appear to be the only other studies in which $\Delta Q_{O_2}(t)$, τ, and Δ[PC](t) were all measured: those of Piiper et al. (1968)

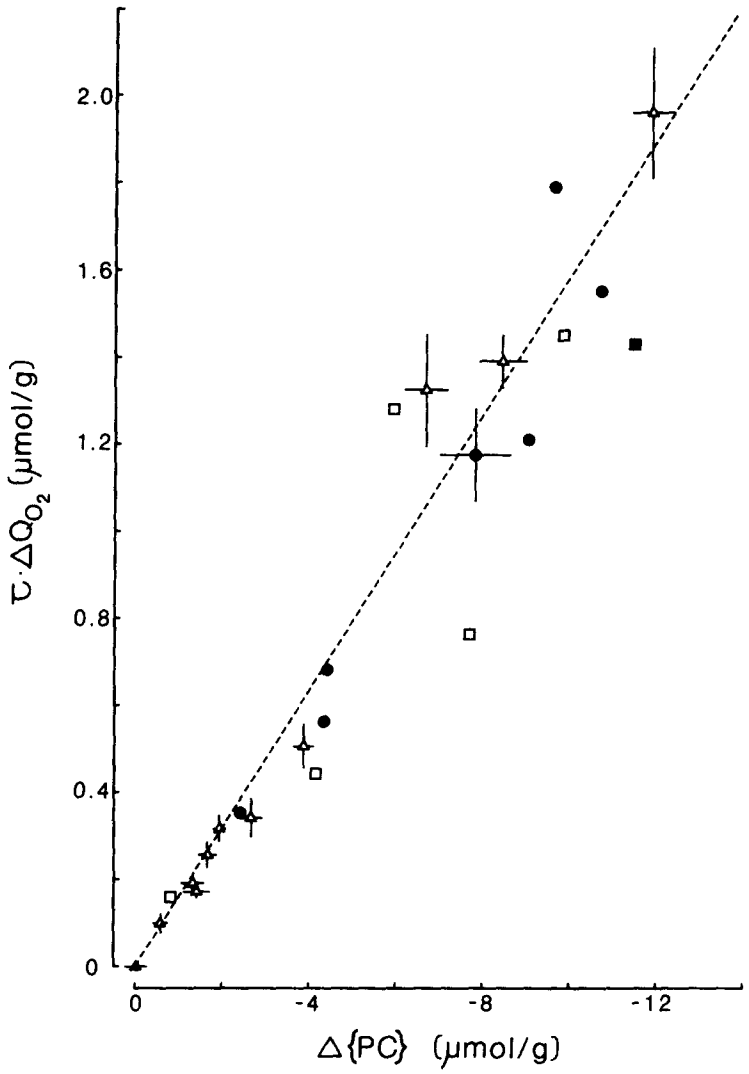

FIGURE 7. Relationships between Δ{PC} and $\tau \cdot \Delta Q_{O_2}$ in the excised frog sartorius at 20°C,[3] in situ dog gastrocnemius at 36°C (Piiper et al., 1968), and perfused cat biceps at 30°C (Kushmerick, M., personal communication). Filled symbols denote steady state values; open symbols indicate non-steady state values. Errors bars designate ± SE. The dashed line is that predicted by Eq. 12, with p = 6.3 mol PC/mol O_2.

[3] The values of ΔQ_{O_2} used here are 10–15% higher than the measured values shown in Figs. 2 and 5. The muscles used for the measurement of ΔQ_{O_2} typically had slightly higher values of $\{C_T\}$ than the muscles used for measurement of Δ{PC} (Mahler et al., 1985). To correct for this, the values of ΔQ_{O_2} were first normalized by $\{C_T\}$ and then multiplied by the mean values of $\{C_T\}$ for the muscles used to measure Δ{PC}.

with the in situ dog gastrocnemius, and Kushmerick and co-workers with the perfused cat biceps (Kushmerick, M., personal communication). Eq. 1 can be rewritten as

$$\tau \cdot \Delta Q_{O_2}(t) \simeq \frac{-1}{p} \Delta\{PC\}(t). \tag{12}$$

The value of τ can be expected to vary widely, depending on the species, muscle type, temperature, and other factors. In fact, it was 2.1–2.6 min in the frog sartorius at 20°C, only 24 s in the dog gastrocnemius at 36°C, and 6.2 min in the cat biceps at 30°C. Similarly, for a given $\Delta\{PC\}$, ΔQ_{O_2} can be expected to vary widely for different muscles. However, the P/O_2 ratio (p) should be quite similar in all cases. Thus, Eq. 12 predicts that for a given $\Delta\{PC\}$, $\tau \cdot \Delta Q_{O_2}$ should be essentially constant in all cases and, more precisely, that it should equal $(-1/p)\Delta\{PC\}$. When plotted in this way (Fig. 7), the data cluster encouragingly around the predicted proportionality. Moreover, in this context, a recent observation by Jacobus and co-workers (Jacobus and Diffley, 1983; Jacobus, 1985) has special significance: they showed that when increases in [creatine] in the medium bathing isolated rat heart mitochondria were accompanied by equal decreases in [PC], as occurs in vivo, steady state Q_{O_2} changed in parallel with [creatine] over the entire physiological range, just as occurs in intact muscle.

Models for the Control of Respiration

A number of quantitative hypotheses for the control of mitochondrial respiration have been advanced, in which the controlling variables include the concentrations of ATP, ADP, and P_i in the surrounding medium (Wilson et al., 1977*b*; Van der Meer et al., 1978; T. L. Hill, 1979; Williamson, 1979; Hansford, 1980; Jacobus et al., 1982). However, only a few attempts have been made to incorporate into these models the extramitochondrial ATPase and CK reactions, which are important determinants of cytoplasmic [ATP], [ADP], and [P_i] in muscle (Chance et al., 1962; Illingworth et al., 1975; Nishiki et al., 1978). Since the model of Chance et al. (1962) was proposed, many additional aspects of the control of respiration in muscle have become apparent, including the chemiosmotic description of oxidative phosphorylation (Mitchell, 1966, 1976), the existence of the adenine nucleotide translocase of the inner mitochondrial membrane and a number of factors controlling the transport of ADP and ATP across this membrane (Vignais, 1976; Klingenberg, 1976, 1980), and the existence of the mitochondrial isozyme of CK (Jacobs et al., 1964), which is bound to the outside of the inner membrane (Scholte et al., 1973). Nevertheless, at least a priori, the assumptions of the model of Chance et al. (1962) remain plausible. The location of CK near the primary cellular source of ATP, as well as its binding near all known extramitochondrial ATPases (for review, see Saks et al., 1978; see also Berson, 1976; Woodhead and Lowey, 1983), is consistent with the assumption of continuous near-equilibrium of CK. The assumption that the Q_{O_2} is determined simply by cytoplasmic [ADP], as postulated in Eq. 6.2, might be considered an extreme oversimplification, since one can expect the Q_{O_2} to be a complicated function of the concentration of O_2 in the matrix, the redox states of the

components of the electron transport chain, the extramitochondrial concentrations of ATP, ADP, and P_i, and the membrane potential and pH gradient across the mitochondrial inner membrane (Wilson et al., 1977*a*, *b*; T. L. Hill, 1979; Williamson, 1979; Hansford, 1980; Klingenberg, 1980; Kushmerick, 1983). On the other hand, many investigators have concluded that oxidative phosphorylation is rate-limited by the delivery of ADP to the matrix by the translocase (for review, see Hansford, 1980; see also Bygrave and Lehninger, 1967; Duee and Vignais, 1969; Kemp et al., 1969; Van der Meer et al., 1978; and for a recent opposing view, see Groen et al., 1982). The rate of inward ADP translocation exhibits a first-order Michaelis-Menten dependence on [ADP] and is competitively inhibited by ATP (Souverijn et al., 1973; Vignais, 1976; Klingenberg, 1976), but since cytoplasmic [ATP] stays essentially constant in muscle under almost any conditions, the rate of translocation would be only implicitly dependent on [ATP], via the K_M for ADP. Thus, it appears that, at least to a good first approximation, Eq. 6.2 is consistent with the hypothesis that the translocase is rate-limiting for oxidative phosphorylation. If that hypothesis is valid, the model of Chance et al. (1962) would appear to be consistent with the recent quantitative formulations of Williamson and co-workers (Illingworth et al., 1975; Williamson, 1979).

The only other attempt to model the control of respiration in intact muscle appears to have been that of Wilson and co-workers (Wilson et al., 1977; Erecinska et al., 1978; Nishiki et al., 1978), who also assumed near-equilibrium of CK, and gave the following expression for Q_{O_2}:

$$Q_{O_2} \simeq \frac{b_1[\text{cyt } c^{2+}]}{b_2 + b_3\,([\text{NAD}]/[\text{NADH}])([\text{ATP}]/[\text{ADP}][\text{P}])^3}, \tag{13}$$

where b_1, b_2, and b_3 are constants.

The in vivo behavior previously used to test these models has been extremely limited. Chance et al. (1962) showed that in isolated mitochondria, the absorbance or fluorescence of NADH or cytochrome *b* closely paralleled the Q_{O_2} and had a K_M for ADP of ~50 μM. They also determined that half-maximal changes in the absorbance or fluorescence of NADH or cytochrome *b* in the intact frog sartorius occurred after four to six twitches and concluded that each twitch caused an increase in [ADP] of ~10 μM. They then calculated the increase predicted by their model, using values of 1/19 for K^* and 35 μM for the resting concentration of ADP (Carlson and Siger, 1959), and arrived at a figure of 6 μM, which was in rough agreement with their estimate based on optical measurements in vivo. However, both estimates are highly parameter-dependent. Using the more current value of 1/166 for K^* (Lawson and Veech, 1979), and values of 37.5 mM, 12 mM, 7.5 mM, and 14.5 μM, respectively, for the resting concentrations of PC, creatine, ATP, and ADP, after a twitch in which 0.5 mM ATP is split, the predicted rise in [ADP] is only 0.8 μM.[4] On the other hand, the

[4] Using the notation introduced above, the amounts of ATP hydrolyzed (y) and resynthesized by CK (x) are related by

$$K^* = (D_0 + y - x)(P_0 - x)/(T_0 - y + x)(C_0 + x), \tag{14}$$

value of K_M used by Chance et al. (1962), 50 μM, was measured in the absence of ATP, a competitive inhibitor of ADP translocation. As discussed below, in the presence of 7.5 mM ATP, a likely in vivo value, K_M probably exceeds 100 μM. The corresponding estimate of the increases in [ADP] based on the in vivo optical measurements thus exceeds 20 μM, which is 25 times larger than that calculated from the assumption of near-equilibrium of CK. As for the model of Wilson and co-workers, the only in vivo behavior successfully predicted appears to have been a range of steady state values of Q_{O_2} in the rat heart (Nishiki et al., 1978).

Use of Eq. 1 to Test Models for the Control of Respiration

A major finding of the present paper is that the relationship between ΔQ_{O_2} and Δ[PC] predicted by the model of Chance et al. (1962) is not consistent with the proportionality between these quantities observed in vivo. Several aspects of this test deserve comment. First, assumption 6.2 is based on steady state measurements; however, the transient state kinetics of the translocase (Pfaff et al., 1969) are evidently rapid enough that only small errors are introduced by making this assumption during non-steady states as well. For example, the integrated form of Eq. 6.2 (Segel, 1976) provides an adequate description of the time course of cytochrome *a* absorbance in isolated mitochondria after the addition of ADP (Chance and Williams, 1956) (Mahler, M., unpublished data). Second, in the derivation of Eq. 5, which gives the predicted relationship between ΔQ_{O_2} and Δ[creatine], the symbols D and T designate the concentrations of MgADP and MgATP, which are true substrates for CK. However, ADP and ATP are transported across the mitochondrial inner membrane in uncomplexed form (Klingenberg, 1976). This can be most easily accounted for by letting K_M designate the apparent K_M of the translocase for MgADP rather than the actual K_M for ADP. The two are related by the factor [MgADP]/[ADP], which can be expected to be practically constant at ~3–10, based on values of $1.3–4.3 \times 10^3$ for the association constant of MgADP (Blair, 1970; De Weer and Lowe, 1973; Lawson and Veech, 1979) and 2.5 mM for $[Mg^{2+}]$ (Wu et al., 1981; cf. Gupta et al., 1980, 1983, who estimate $[Mg^{2+}] < 1$ mM). As for the expected value of this apparent K_M, since ATP competes with ADP for translocation, the K_M for ADP is given by $K_S(1 + [ATP]/K_I)$. Values in the 1–12-μM range have been reported for K_S (Kemp et al., 1969; Souverijn et al., 1973; Klingenberg, 1976). Data on K_I are sparse, but for conditions in which ATP was practically all complexed with Mg, Davis and Lumeng (1975) reported an apparent K_I for MgATP of 0.725 mM in rat liver mitochondria. In muscle, where [MgATP] appears to be ~7.5 mM, $[ATP]/K_I$ = [MgATP]/(apparent K_I) would be of the order of 10, and the apparent K_M of the translocase for MgADP would be 30–100 times K_S. Finally, as discussed and demonstrated above, given an essentially constant value of [ATP], the first-order kinetics of ΔQ_{O_2} and the proportionality between ΔQ_{O_2} and Δ[creatine] expressed in Eq. 1 are logically equivalent properties. Because

which leads to the quadratic equation $ax^2 + bx + c = 0$, with $a = 1 - K^*$, $b = K^*(y - C_0 - T_0) - y - P_0 - D_0$, and $c = y(P_0 + K^*C_0)$. Using the parameter values chosen above for the frog sartorius at 20°C, for $y = 0.5$ mM, $x = 0.4992$ mM. In general, $x/y \simeq 0.998$.

the assumption of near-equilibrium of CK ensures that the value of [ATP] predicted by the model stays virtually constant, the curvilinear relationship between ΔQ_{O_2} and Δ[creatine] predicted by the model implies that the predicted kinetics of ΔQ_{O_2} must be markedly different from the pseudo–first-order behavior actually observed. This has in fact been demonstrated explicitly (Whipp and Mahler, 1980).

For the remaining published model for the control of respiration in muscle, that of Wilson and collaborators (Wilson et al., 1977*a*; Nishiki et al., 1978), the derivation of exact expressions for the predicted time course of Q_{O_2} following a step or pulse change in the rate of ATP hydrolysis is problematical because of the complex and highly nonlinear nature of the describing equations. A numerical solution is probably feasible, but it seems a priori unlikely that the required pseudo–first-order behavior would result. The predicted relationship between Δ[creatine] and ΔQ_{O_2} is somewhat more tractable, but also appears highly nonlinear. It seems fair to conclude that the modeling of the control of respiration in muscle is still in a rudimentary stage.

It is worth noting that because of the apparent proportionality between $\Delta\{P_i\}$ and ΔQ_{O_2} (Eq. 4), the first-order kinetics of ΔQ_{O_2} would be accounted for if respiration were rate-limited by P_i transport across the mitochondrial inner membrane and this rate were proportional to $[P_i]$ over the physiological range. However, studies with intact mitochondria have shown that this transport rate has a Michaelis-Menten dependence on $[P_i]$, with $K_M \simeq 1$ mM (Chance and Connelly, 1957; Coty and Pedersen, 1974). Because the normal proportionality between $\Delta[P_i]$ and ΔQ_{O_2} extends to values of $\Delta[P_i]$ of 15–20 mM (Figs. 6 and 7), this proportionality is evidently coincidental, not causal. (For additional evidence, see Chance et al., 1982.)

In order to quantify the control exerted by a given reaction in a metabolic pathway on the flux (J) through the pathway, Kacser and Burns (1973) and Heinrich and Rapoport (1974) devised the concept of control strength (C), defined as the fractional change in flux induced by a given fractional change in the concentration of the enzyme (E) catalyzing the reaction under consideration, i.e.,

$$C = \frac{\partial J}{\partial E} \cdot \frac{E}{J}. \tag{15}$$

This methodology was used by Groen et al. (1982) to analyze the control of respiration in isolated mitochondria, but it is not particularly useful in the present context. It applies only to steady states (Kacser and Burns, 1973), for which case some consideration (compare Fig. 1 above with Fig. 8 of Groen et al.) shows that the extramitochondrial ATPase reaction has control strength 1.0, while all other reactions should have control strength zero. This simply restates the obvious fact that during steady states the rate of ATP production via oxidative metabolism exactly matches the rate of ATP utilization.

Role of Mitochondrial Creatine Kinase in the Control of Respiration

PSEUDO–FIRST-ORDER KINETICS OF MITOCHONDRIAL CK The observed proportionality between $\Delta\{PC\}$ and ΔQ_{O_2} during recovery, together with the

constancy of {ATP}, has an interesting implication, stated in Eq. 16. The forward CK reaction, which presumably occurs within the mitochondria (Fig. 1; Jacobus and Lehninger, 1973), must be pseudo–first order in Δ{creatine}, with rate constant $1/\tau$:

$$\Delta v_{CK} \simeq \frac{1}{\tau}\,\Delta\{\text{creatine}\} = \frac{-1}{\tau}\,\Delta\{\text{PC}\}. \tag{16}$$

In this equation, v_{CK} denotes the net (forward) rate of the CK reaction, and Δv_{CK} denotes the change in v_{CK} from its basal value. To deduce Eq. 16, consider first the arguments summarized in Eq. 17.

$$\Delta v_{CK} \simeq \frac{d}{dt}\,\Delta\{\text{PC}\} \simeq \begin{matrix}\text{suprabasal}\\ \text{rate of ATP}\\ \text{utilization}\end{matrix} \simeq \begin{matrix}\text{suprabasal}\\ \text{rate of ATP}\\ \text{production}\end{matrix} \simeq p \cdot \Delta Q_{O_2}. \tag{17}$$

Because {ATP} stays fixed, the suprabasal rates of ATP production and utilization must be closely matched at all times. As stated in the rightmost portion of Eq. 17, of the pathways for suprabasal ATP production, all but oxidative metabolism can be ignored. The rate of ATP production by the reverse CK reaction (Fig. 1) is normally virtually identical to the rate of ATP hydrolysis, as shown by the constancy of {ATP} during a tetanus (see Introduction), but because suprabasal ATP hydrolysis during recovery appears to be negligible by the time ΔQ_{O_2} becomes monoexponential (Mahler et al., 1985), suprabasal ATP production via extramitochondrial CK is presumably also negligible. Similarly, the fraction of ATP production coupled to lactate production appears to be no more than 5% (Kushmerick and Paul, 1976). As for the pathways for ATP utilization, as just noted, suprabasal hydrolysis of ATP appears to be negligible, and the only other quantitatively important sink for ATP is the forward CK reaction. As stated in the left-hand portion of Eq. 17, suprabasal ATP production during the period of interest thus results in a virtually stoichiometric increase in {PC}. Eq. 17 shows that during recovery, ΔQ_{O_2} provides an indirect measure of Δv_{CK}, and substituting the observed proportionality between Δ{PC} and ΔQ_{O_2} (Eq. 1) into the rightmost term of Eq. 17 yields Eq. 16.

TWO HYPOTHESES CONCERNING MITOCHONDRIAL CK The observation that the forward CK reaction, which will be assumed to occur in the mitochondria, obeys a pseudo–first-order rate law during recovery leads to two hypotheses, one conventional and unenlightening, the other unconventional but quite provocative. Note first that the close match between the mitochondrial CK rate and the rate of ATP production by oxidative metabolism (Eq. 17) is consistent with the conventional assumption that the mitochondrial CK reaction is rate-limited by oxidative metabolism. It would then follow immediately from the first-order kinetics of ΔQ_{O_2} that Δv_{CK} must also exhibit apparent first-order kinetics (Eq. 16), and that Δ{PC} must change in proportion to ΔQ_{O_2} (Eq. 1). With this hypothesis, however, one is still faced with the task of accounting for the first-order kinetics of ΔQ_{O_2}, which, as discussed above, appears to be a formidable one.

The close match between Δv_{CK} and $p \cdot \Delta Q_{O_2}$ suggests an alternative hypothesis: that oxidative metabolism, rather than being rate-limiting for the mitochondrial

CK reaction, is rate-limited by it. This would neatly explain the first-order kinetics of muscle ΔQ_{O_2}, since the mitochondrial CK reaction is observed to be pseudo–first order in Δ{creatine} (Eq. 16). However, this hypothesis has at least two immediate corollaries that fly in the face of conventional wisdom. First, since a metabolic pathway cannot be rate-limited by a near-equilibrium reaction (Wilson et al., 1974), it is implied that, contrary to a widely made assumption,

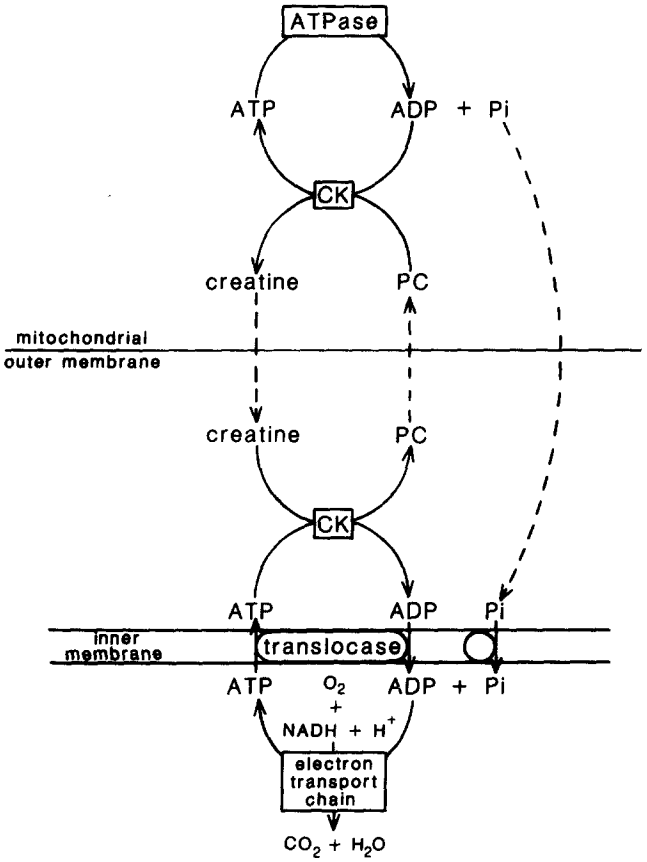

FIGURE 8. Modification of Fig. 1 consistent with the present results, which suggest that respiration in muscle may be rate-limited by the production of ADP via mitochondrial CK, and that fluxes of ADP and ATP between extramitochondrial ATPases and mitochondria are therefore negligible.

the mitochondrial CK reaction cannot be continuously near equilibrium. Second, if the intramitochondrial production of ADP via CK is an obligatory step in the coupling between ATP hydrolysis and oxidative phosphorylation, it follows that the direct flux of ADP from extramitochondrial ATPases to the translocase must be negligible (Fig. 8); in other words, this hypothesis for the control of respiration has as a direct corollary the controversial "creatine shuttle" hypothesis (first proposed by Gerlach, 1967, and Gercken and Schlette, 1968; for reviews, see Saks et al., 1978; Jacobus and Ingwall, 1980; Brautbar and Bessman, 1985), so

called because it postulates that essentially all the high-energy phosphate and phosphate acceptor that travels between cellular sites of ATP production and utilization is in the form of PC and creatine rather than ATP and ADP. At least one aspect of this latter prediction appears plausible from first principles. If it is assumed that the CK reaction stays near equilibrium in a well-stirred cytoplasm, it can be calculated that in the frog sartorius ~99.8% of the ADP produced by extramitochondrial ATPases is rephosphorylated by CK.[4] For example, for an ATP splitting of 5 mM, the calculated Δ[ADP] would be only 9 μM, which is less than the calculated resting [ADP] (≃15 μM), and probably very much smaller than the apparent K_M of the translocase for MgADP, which is likely to be of the order of 100 μM in vivo (see above). Moreover, because CK is bound close by these ATPases (for review, see Saks et al., 1978; see also Berson, 1976; Woodhead and Lowey, 1983), it is possible that near the active sites of this CK the concentration of ADP is considerably higher, and that of ATP considerably lower, than in a well-stirred medium (for review, see Saks et al., 1978; also see Yagi and Mase, 1965; Bessman et al., 1980; Grosse et al., 1980; Spitzer et al., 1981), so that the rephosphorylation of ADP at these sites goes even further toward completion. Finally, the diffusion coefficient for ADP through the cytoplasm is apparently appreciably less than that of creatine (Yoshizaki et al., 1982; Meyer et al., 1984), so that the flux of ADP from ATPases to mitochondria should be less, relative to that of creatine, than the flux calculated assuming a well-stirred medium. Taken together, these observations suggest that the direct flux of ADP to the mitochondria may well be so small as to have only a negligible effect on the Q_{O_2}, as predicted by the hypothesis that respiration in muscle is rate-limited by the mitochondrial CK.

As for the prediction that the mitochondrial CK reaction is not continuously near equilibrium, ^{31}P NMR spin transfer measurements, which yield unidirectional rates of phosphate transfer between ATP and PC, have recently been made in intact skeletal muscles and hearts (for review, see Meyer et al., 1982; see also Nunnally, 1980; Matthews et al., 1981, 1982) and used to test the hypothesis that the CK reaction is near equilibrium. The simplest but least realistic way of analyzing these results is to assume that the tissue can be considered a well-stirred solution. When this is done, some results are consistent with near-equilibrium of CK, but others are not. In either case, these interpretations appear to be highly model-dependent, and this model ignores evidence from several laboratories which suggests strongly that the concentrations of ATP and ADP at the active sites of mitochondrial CK may be markedly different, perhaps 5- to 10-fold, from those in the cytoplasm (Saks et al., 1976, 1980; Yang et al., 1977; DeFuria et al., 1980; Erickson-Viitanen et al., 1982*a*, *b*; Moreadith and Jacobus, 1982). As discussed above, the concentrations of ATP and ADP at the active sites of the CK bound near ATPases may also be appreciably different from the average concentrations in the cytoplasm. Evidently, models postulating two (Nunnally and Hollis, 1979; Nunnally, 1980), three, or more CK sites and incorporating their spatial relationships with ATPases and the translocase are needed. Unfortunately, it seems likely that any given set of whole-muscle or whole-cell ^{31}P NMR spin transfer data might be consistent with several such models. On the other hand, this technique does appear to be uniquely suited to

determining whether CK is near equilibrium during steady and non–steady states in isolated mitochondria.

What Is Meant by a Creatine Shuttle?

The testing and discussion of the creatine shuttle hypothesis have been handicapped by the lack of a precise statement of it. It is generally understood to postulate that essentially all transport of high-energy phosphate and phosphate acceptor occurs via PC and creatine; unfortunately, there has been no accepted quantification of "essentially all," and this has caused confusion. For example, with models that assume that the CK reaction stays near equilibrium in a well-stirred cytoplasm, if it is further assumed that the equilibrium-restoring response to the utilization or production of ATP occurs at the CK sites contiguous to the disturbing reactions, then, as just discussed, it can be calculated that the fractions of high-energy phosphate and phosphate acceptor flux occurring via PC and creatine are as great as 0.998. Similar conclusions with regard to several such models have been reached by others (Garfinkel and Kohn, 1980; Wilson et al., 1981), who have considered these models consistent with the idea of a creatine shuttle. However, discussion of the shuttle hypothesis by its proponents shows that a key element in it is the proposal that, outside the matrix, changes in [ATP] and [ADP] are largely restricted to microenvironments near the ATPases and translocase, because of the juxtaposition of bound CK. An important ancillary hypothesis is that changes in the rates at which ATP is delivered to the ATPases, and ADP to the translocase, are triggered by changes in cytoplasmic [PC] and [creatine] (Gercken and Schlette, 1968; Saks et al., 1974, 1976, 1978, 1980; Jacobus, 1980; Bessman and Geiger, 1981; Moreadith and Jacobus, 1982; Jacobus and Diffley, 1983). In this context, it is clear that no model postulating a well-stirred cytoplasm can be consistent with the creatine shuttle hypothesis.

In order for the direct flux of ADP from ATPases to the mitochondria to have a negligible effect on the Q_{O_2}, creatine must account for no less than ~99.95% of the high-energy phosphate acceptor flux to the mitochondria, depending on the in vivo K_M of the translocase for ADP. Since in vivo transport of ADP, ATP, creatine, and PC does not appear to be readily measurable, much less with the three- or four-digit accuracy necessary to distinguish among the predictions of this and other models, future discussion and experimental tests of the creatine shuttle hypothesis could more profitably focus not on phosphate transport per se, but on the equivalent problem of quantifying the extent to which changes in the Q_{O_2} are triggered, via mitochondrial CK, by changes in cytoplasmic [creatine] and [PC] rather than by changes in cytoplasmic [ADP] (and/or [P_i]). In this case, the predictions of the shuttle hypothesis and of models postulating a well-stirred cytoplasm are mutually exclusive: a "creatine trigger" mechanism is predicted in the former case to account for all of the change in Q_{O_2} and, in the latter case, for none of it.

Future Directions

For an improved understanding of the control of respiration in muscle, the most urgent need would now appear to be not new data, but the establishment of a satisfactory context for the interpretation of past and future experiments by the

creation of realistic models. They should be space-dependent (Meyer et al., 1984), with multiple sites for CK and variable distances between CK and the sinks and sources for ATP, to allow for the possibility of physical juxtaposition and functional coupling as bi-enzyme complexes. Until stronger evidence is presented for continuous near-equilibrium of CK, its rapid-equilibrium random-reaction mechanism (Watts, 1973; Saks et al., 1975) should be incorporated. Ideally, these models would consider the four coupled transport systems of the mitochondrial inner membrane (T. L. Hill, 1979), not just that of ADP and ATP, and would describe non-steady as well as steady states. It seems likely that only after the predictions of such sophisticated models become available will it be possible to elucidate the mechanisms underlying the pseudo-first-order kinetics of ΔQ_{O_2} and the proportionality between Δ[creatine] or Δ[PC] and ΔQ_{O_2}.

APPENDIX

Derivation of Eq. 1

A formal derivation of Eq. 1 for the case of the impulse response shown in Figs. 2 and 4 is the following. Let T denote the time at which $\Delta Q_{O_2}(t)$ becomes monoexponential. Then for any $t \geq T$,

$$\Delta Q_{O_2}(t) = \Delta Q_{O_2}(T)e^{-(t-T)/\tau}. \tag{A1}$$

As discussed in the text and stated in Eq. 17,

$$p \cdot \Delta Q_{O_2}(t) \simeq \frac{d}{dt}\Delta[PC](t), \tag{A2.1}$$

or, rearranging,

$$\Delta Q_{O_2}(t) \simeq (1/p)\frac{d}{dt}\Delta[PC](t). \tag{A2.2}$$

Equating the right-hand sides of Eqs. A1 and A2.2 and integrating from t to ∞ yields Eq. 1; the former integral is $-\tau \cdot \Delta Q_{O_2}(t)$, and the latter is $(1/p)\Delta[PC](t)$.

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