The Relationship between Initial Creatine Phosphate Breakdown and Recovery Oxygen Consumption for a Single Isometric Tetanus of the Frog Sartorius Muscle at 20°C

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ABSTRACT   A previous paper (Mahler, M. 1978. *J. Gen. Physiol.* **71:**559–580) describes the time-course of the suprabasal rate of oxygen consumption (ΔQo_2) in the sartorius muscle of *R. pipiens* after isometric tetani of 0.1–1.0 s at 20°C. To test whether these were the responses to impulse changes in the rate of ATP hydrolysis, we compared the total suprabasal oxygen consumption during recovery ($\Delta[O_2]$) with the amount of ATP hydrolyzed during a contraction, measured indirectly as the decrease in creatine phosphate ($\Delta[CP]_0$). If suprabasal ATP hydrolysis during recovery is negligible in comparison with that during contraction, $\Delta[CP]_0/\Delta[O_2]$ should approximate the $P:O_2$ ratio for oxidative metabolism, which has an expected value of 6.1–6.5. We found:

	Tetanus duration		
		s	
	0.2	0.5	1.0
$\Delta[CP]_0$, $\mu mol/g$	1.44±0.25 (11)	2.98±0.30 (9)	4.31±0.24 (15)
$\Delta[O_2]$, $\mu mol/g$	0.214±0.021 (12)	0.436±0.039 (7)	0.698±0.028 (8)
$\Delta[CP]_0/\Delta[O_2]$	6.72±1.36	6.83±0.90	6.18±0.43

All values are ± SEM. Number in parentheses is number of measurements.

We conclude that in this muscle at 20°C: (*a*) after a tetanus of 0.2–1.0 s, $\Delta Qo_2(t)$ can be considered the response to an impulse increase in the rate of ATP hydrolysis; (*b*) the reversal during recovery of unidentified exothermic reactions occurring during the contraction (Woledge, R. C. 1971. *Prog. Biophys. Mol. Biol.* **22:**39–74) can be coupled to an ATP hydrolysis that is at most a small fraction of $\Delta[CP]_0$; (*c*) the pooled mean for $\Delta[CP]_0/\Delta[O_2]$, 6.58 ± 0.55, sets an experimental lower bound for the $P:O_2$ ratio in vivo.

INTRODUCTION

The preceding paper in this series (Mahler, 1978 *b*) outlines the rationale for a systems approach to the study of respiratory control in the intact frog sartorius muscle, and describes the time-course of the rate of oxygen consumption (Qo_2) by this muscle after single isometric tetani of 0.1–1.0 s at 20°C. It was intended

that these kinetics of Qo_2 would be the responses to a family of impulse-like[1] changes in the rate of ATP hydrolysis[2] by the muscle, and the experiments described here were designed to test whether this was the case.

During contraction at 20°C, the rate of ATP hydrolysis in the frog sartorius increases drastically (Canfield and Marechal, 1973; cf. Discussion). Moreover, on the time scale of oxidative recovery (Mahler, 1978 b), a contraction lasting on the order of 1 s is practically instantaneous. It follows that the time-course of change in the rate of ATP hydrolysis will satisfactorily approximate an impulse if, after the relaxation of tension, this rate returns to, or close to, its basal level, and remains there during oxidative recovery, so that the total suprabasal ATP hydrolysis during recovery is small in comparison with that during the contraction. To test whether this condition was satisfied, we compared the total suprabasal oxygen consumption during recovery ($\Delta[O_2]$) with the amount of ATP hydrolyzed during a tetanus, measured indirectly as the decrease in creatine phosphate ($\Delta[CP]_0$). If suprabasal ATP hydrolysis during recovery is negligible in comparison with $\Delta[CP]_0$, the ratio $\Delta[CP]_0/\Delta[O_2]$ should approximate the $P:O_2$ ratio for oxidative metabolism (cf. Discussion for details of this argument).

In vitro reconstruction of oxidative metabolism has furnished the conclusion that the $P:O_2$ ratio associated with the complete oxidation of glycogen is 6.2–6.5, and with the oxidation of free fatty acids (FFA), ∼ 5.6 (McGilvery, 1975; Harper et al., 1977). The corresponding respiratory quotients are 0.7 and 1.0, respectively. The respiratory quotient for suprabasal oxidative metabolism in frog skeletal muscle is in the range 0.9–1.0 (Fenn, 1927; Gemmilll, 1936; Hill, 1940), and since the substrates are presumably glycogen and FFA, it follows that the expected $P:O_2$ ratio lies in the range 6.1–6.5. Assuming that the $P:O_2$ ratio in vivo does in fact have the value predicted by this classical mapping of oxidative metabolism (cf. Discussion), a value in or near the range 6.1–6.5 for $\Delta[CP]_0/\Delta[O_2]$ would thus imply that the time-course of ATP hydrolysis during and after a single tetanus was well described as an impulse. We found that for tetani of 0.2–1.0 s, $\Delta[CP]_0/\Delta[O_2]$ had a pooled mean value of 6.58 ± 0.55.

MATERIALS AND METHODS

Measurement of $\Delta[CP]_0$

In order to measure $\Delta[CP]_0$, sartorii of Rana pipiens were frozen after a tetanus with the immersion apparatus developed in this laboratory (Mommaerts and Schilling, 1964). After dissection, muscles were kept for a minimum of 2 h at 4°C in Ringer solution bubbled with 95% O_2, 5% CO_2. It was intended that before stimulation, a muscle be in a resting steady state at 20°C. Accordingly, after a muscle had been mounted on the

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[1] If $p(t)$ denotes a pulse of duration w, amplitude A/w, and area A, where t denotes time, the impulse function of area A is defined as $\lim_{w \to 0} p(t)$. Strictly speaking, such functions, while mathematically convenient, are not physically realizable; however, they can often be satisfactorily approximated by wave-forms whose amplitude is large near $t = 0$, and negligible at all other times.

[2] ATP hydrolysis designates the ATPase-catalysed reaction: $ATP \rightarrow ADP + Pi + H_2O +$ (energy for contraction, ion pumping, etc.), as distinguished from other ATP-utilizing reactions, such as those catalyzed by creatine kinase or adenylate kinase.

stimulating grid, with its length set at the value measured in situ, it was immersed in a thermos flask filled with oxygenated Ringer solution at 20°C for 30 min; this equilibration period was sufficiently long to allow ΔQ_{O_2} to reach its basal level (cf. Mahler, 1978 b). The Ringer-filled thermos was then removed, the muscle chamber was lowered to enclose the muscle, and a thermos was filled with isopentane slush mounted beneath the chamber. 90 s after being enclosed in the chamber, an experimental muscle was stimulated supramaximally for either 0.2, 0.5, or 1.0 s, with stimulus duration 0.6 ms and frequency 70/s, and immersed in the isopentane 0.4 s after stimulation had ceased. This allowed the muscle to relax completely before immersion. The paired control muscle was simply frozen 90 s after enclosure in the chamber. Muscles were weighed after freezing. Levels of creatine (Cr), CP, and ATP were measured by the methods of Homsher et al. (1972). $\Delta[CP]_0$ was calculated as the average of the increase in [Cr] ($\Delta[Cr]$) and the decrease in [CP] ($\Delta[CP]$).

Because CP splitting during a tetanus has a high Q_{10}, temperature control was critical in these experiments. After the muscle was removed from the Ringer-filled thermos at 20°C, its temperature must have quickly reached the room level, which was 22–23°C; it was necessary to ensure that it again reached 20°C before stimulation. The temperature in the muscle chamber could be controlled in two ways: by rapid circulation of glycerol through coils in the walls of the chamber, and by the circulation of gas through the chamber walls and the chamber itself. In preliminary experiments, a combination of glycerol temperature and gas flow was found which would bring the chamber temperature to within 0.2° of 20°C within 30–45 s after the chamber was closed. The temperature then oscillated with an amplitude of ~0.15°, and mean within 0.1° of 20°C. Since the temperature within a muscle of the size used in these experiments will uniformly match a constant external temperature within ~10 s, it can be concluded that the muscle temperature was very close to 20°C during stimulation.

Measurement of $\Delta[O_2]$

$\Delta[O_2]$ was measured as the integral over the entire recovery period of $\Delta Q_{O_2}(t)$, the suprabasal rate of oxygen consumption during recovery (cf. Fig. 2). The technique for measuring $\Delta Q_{O_2}(t)$ was described in an earlier paper (Mahler, 1978 b), and the results presented here were for the most part derived from experiments already described there. However, those measurements were made on a different batch of frogs than the measurements of $\Delta[CP]_0$. To check that $\Delta Q_{O_2}(t)$ was not markedly batch-dependent, six additional measurements were made of $\Delta Q_{O_2}(t)$ after a 0.2-s tetanus, with frogs from the same batch used for measurement of $\Delta[CP]_0$. The chamber gas composition was in five experiments 39.5% O_2, 2.1% CO_2, and 58.4% N_2, and in one case 95% O_2, 5% CO_2. After these measurements of $\Delta Q_{O_2}(t)$, as well as those described in the preceding paper, muscles were frozen by immersion in liquid N_2, and analyzed for total creatine (C_T); in a few early experiments, however, this was not done (cf. Table II). Integration of $\Delta Q_{O_2}(t)$ was done by the trapezoidal rule. The basal level of ΔQ_{O_2} during recovery was taken to be the value observed at the end of recovery; the effect of this assumption on $\Delta[CP]_0/\Delta[O_2]$ is considered under Results.

Statistical Analysis

To compute the confidence limits on $\Delta[CP]_0/\Delta[O_2]$, two procedures were used. The first was that given by Bliss (1967), according to which the confidence limits on a ratio $\bar{X}/\bar{Y} = \hat{R}_*$ are given by:

$$C\hat{R}_* \pm \sqrt{(C-1)\left(C\hat{R}_* + \frac{V_X}{V_Y}\right)}, \tag{1}$$

where V_X and V_Y are the sample variances for X and Y,

$$C \equiv \bar{Y}^2 / (\bar{Y}^2 - V_Y \cdot t^2),$$ (2)

and t is the value of Student's t for the number of degrees of freedom:

$$n = (V_X + \hat{R}_*^2 \cdot V_Y)^2 / \left(\frac{V_X^2}{n_X} + \frac{\hat{R}_*^4 \cdot V_Y^2}{n_Y} \right).$$ (3)

This procedure is evidently the most accurate for a single ratio, but it is not clear how it can be used to pool results. When V_Y is sufficiently small, however, the confidence limits given by Eq. 1 can be closely approximated by assuming that the true mean X/Y is normally distributed, with variance given by:

$$\frac{V\left(\dfrac{\bar{X}}{\bar{Y}}\right)}{\left(\dfrac{\bar{X}}{\bar{Y}}\right)^2} = \frac{V_X}{\bar{X}^2} + \frac{V_Y}{\bar{Y}^2},$$ (4)

and n given by Eq. 3. If this approximation is accurate, results can then be pooled in the usual way. In the present case, for a single tetanus duration, the confidence limits on $\Delta[CP]_0/\Delta[O_2]$ calculated via Eqs. 3 and 4 agreed to within 5% with the more exact limits based on Eqs. 1-3. Accordingly, the value for different tetanus durations were pooled, using:

$$V_{(\bar{X}+\bar{Y}+\bar{Z}/3)} = \sqrt{(V_{\bar{X}}^2 + V_{\bar{Y}}^2 + V_{\bar{Z}}^2)/3}$$ (5)

and

$$n = (V_X + V_Y + V_Z)^2 / \left(\frac{V_X^2}{n_X} + \frac{V_Y^2}{n_Y} + \frac{V_Z^2}{n_Z} \right).$$ (6)

These are the formulae given by Bliss (1967) for pooling means with unequal variances. The standard errors given in Table II have been placed in parentheses to indicate that they are approximate.

RESULTS

Experimental Results

The chemical results are summarized in Table I. The mean resting value of $[CP]/[C_T]$, 0.730, is within the range of values reported at 0°C, but somewhat lower than their average, which is about 0.80 (Wilkie, 1968; Homsher et al., 1975). The peak tetanus tension averaged 2.71 kg/cm^2, ~35% higher than that typically observed at 0°C, and in agreement with published values at 20°C (Hill, 1951; Hill and Woledge, 1962; Canfield and Marechal, 1973). For each tetanus duration, $\Delta[ATP]$ was small, and not statistically different from zero. As shown in Fig. 1, the relationship between $\Delta[CP]_0$ and tetanus duration was curvilinear, consistent with results obtained at 0°C (Woledge, 1971; Homsher et al., 1975; Kushmerick and Paul, 1976 b).

$\Delta Q_{O_2}(t)$ after a 0.2-s tetanus was well fit by the general equation given previously (Mahler, 1978 b):

$$\Delta Q_{O_2}(t) = Q_0 + Q_1(e^{-k_1 t} - e^{-k_2 t}),$$ (7)

(cf. Fig. 2), with Q_0, Q_1, and k_1 and k_2, as well as the total recovery oxygen consumption, $\Delta[O_2]$, having mean values not different from those previously observed ($P > 0.4$ in each case). Accordingly, the two sets of results for 0.2-s tetani were pooled in the analysis described below.

For the experiments of this and the previous paper, $\Delta[O_2]$ showed a curvilinear dependence on tetanus duration, similar to that of $\Delta[CP]_0$ (cf. Fig. 3). The relationship between $\Delta[CP]_0$ and $\Delta[O_2]$ is described in Table II and Fig. 4. Direct computation of $\Delta[CP]_0/\Delta[O_2]$ gave values that were generally slightly higher than the range 6.1–6.5, but in no case was $\Delta[CP]_0/\Delta[O_2]$ statistically different from any of these values ($P > 0.4$ for individual values and pooled mean).

TABLE I

RESULTS OF CHEMICAL ANALYSES ON QUICK-FROZEN MUSCLES

	Tetanus duration		
	s		
	0.2	0.5	1.0
	Number of measurements		
Analysis	11	9	15
Resting [Cr], $\mu mol/g$	9.72±0.53	9.01±0.53	8.71±0.67
Resting [CP], $\mu mol/g$	24.36±0.33	23.64±0.48	24.33±0.38
Resting $[CP]/[C_T]$, $\mu mol/\mu mol$	0.720±0.010	0.725±0.009	0.740±0.015
$\Delta[Cr]$, $\mu mol/g$	1.40±0.21	3.07±0.33	4.24±0.30
$\Delta[CP]$, $\mu mol/g$	1.48±0.54	2.89±0.54	4.39±0.49
$\Delta[CP]_0$, $\mu mol/g$	1.44±0.25	2.98±0.29	4.31±0.24
$\Delta[CP]_0/[C_T]$, $\mu mol/\mu mol$	0.046±0.008	0.092±0.007	0.130±0.007
$P_{max} \cdot l_o/M$, kg/cm^2	2.60±0.08	2.88±0.07	2.69±0.06

$[C_T]$ denotes the total creatine concentration ([Cr] + [CP]). All values are ± SEM.
M (last line) denotes muscle weight.

On average, the peak tension per cross-sectional area, expressed as $P_{max} \cdot l_o/[C_T]$ (Carlson, 1963; Wilkie, 1968), was slightly greater in the contractions for which $\Delta[CP]_0$ was measured than in those preceding the measurement of $\Delta[O_2]$. It is sometimes assumed that for a given tetanus duration, $\Delta[CP]_0$ is proportional to $P_{max} \cdot l_o/[C_T]$ (e.g., cf. Woledge, 1971), and the same might be expected to be true for $\Delta[O_2]$. The present data provide no evidence for such relationships. For example, the correlation coefficients between $P_{max} \cdot l_o/[C_T]$ and $\Delta[O_2]$ for tetani of 0.2, 0.5, and 1.0 s were 0.05, −0.40, and −0.19, respectively; $\Delta[O_2]$ varied over roughly a 1.5-fold range in each case. It is of course possible that the absence of a strong positive correlation between $P_{max} \cdot l_o/[C_T]$ and $\Delta[CP]_0$ results from a lack of precision in the measurement of the latter parameters. If $\Delta[CP]_0$ and $\Delta[O_2]$ were normalized by $P_{max} \cdot l_o/[C_T]$, $\Delta[CP]_0/\Delta[O_2]$ was within the range 5.8–6.1 for each tetanus duration, with pooled mean 5.91 ± 0.54 (cf. Table II).

For the calculation of $\Delta[O_2]$, the basal level of Q_{O_2} was assumed to be that observed at the end of recovery, $(\Delta Q_{O_2})_b$ (cf. Mahler, 1978 *b*), rather than that before the contraction. If the latter value was used instead, the results were not

fundamentally different. Over 30 min, the average area under $(\Delta Q_{O_2})_b$ for tetani of 0.2, 0.5, and 1.0 s were -0.24, 0.90, and 3.12 $\mu l/g$, respectively, $\sim$ -4.6%, 8.6%, and 18.6% as large as the calculated values of $\Delta[O_2]$. If $\Delta[O_2]$ is changed by these amounts, the values of $\Delta[CP]_0/\Delta[O_2]$ became 7.0, 6.3, and 5.2, respectively (mean 6.2); if normalized for tension, the values were 6.2, 5.6, and 4.9, with a mean of 5.5.
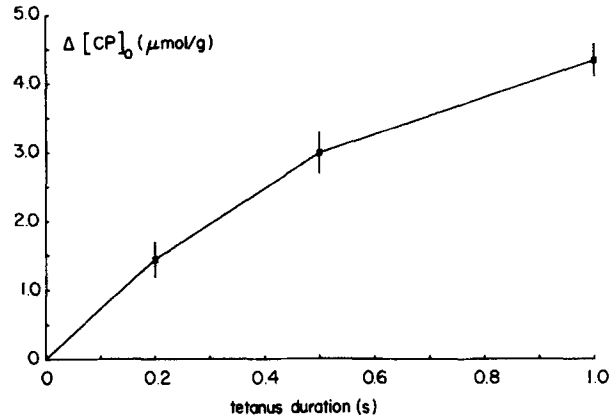

FIGURE 1. The relationship between the duration of an isometric tetanus and the decrease in [CP] during the tetanus. Bars denote $\pm$ 1 SEM.
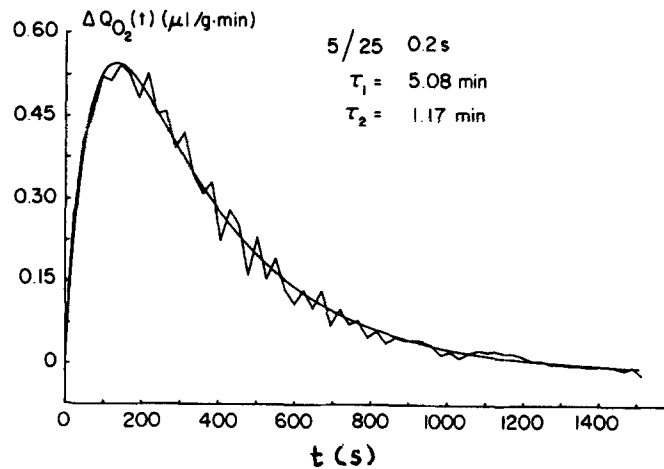

FIGURE 2. Typical fit of $\Delta Q_{O_2}(t)$ after an isometric tetanus of 0.2 s by the function $Q_0 + Q_1(e^{-k_1 t} - e^{-k_2 t})$.

DISCUSSION

Relationship between $\Delta[CP]_0/\Delta[O_2]$ and the $P:O_2$ Ratio

The relationship between $\Delta[CP]_0/\Delta[O_2]$ and the $P:O_2$ ratio for oxidative metabolism in vivo can be derived in the following way:

$P : O_2$ ratio

$$\simeq \frac{\text{total suprabasal ATP production during recovery}}{\text{total suprabasal } O_2 \text{ consumption during recovery } (\Delta[O_2])} ; \qquad (8)$$

$$\simeq \frac{\text{total suprabasal ATP utilization during recovery}}{\Delta[O_2]} ; \qquad (8.1)$$

$$\simeq \frac{\Delta[CP]_0 + \text{total suprabasal ATP hydrolysis during recovery}}{\Delta[O_2]} . \qquad (8.2)$$

It follows from Eq. 8.2 that $\Delta[CP]_0 / \Delta[O_2]$ can closely approximate the $P : O_2$ ratio only if the amount of suprabasal ATP hydrolysis during recovery is very small in comparison with $\Delta[CP]_0$. This is the same condition that must be fulfilled if the time-course of change in the rate of ATP hydrolysis is to approximate an impulse (cf. Introduction).
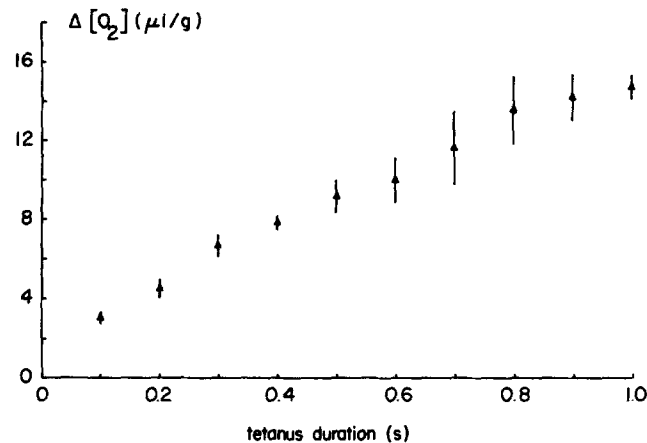

FIGURE 3.   The relationship between the duration of an isometric tetanus and the total suprabasal oxygen consumption after the tetanus, $\Delta[O_2]$. Bars denote $\pm$ 1 SEM.

Eq. 8 is based on the definition of the $P : O_2$ ratio, and on the assumption that essentially the entire net suprabasal ATP production occurs via the oxidative pathway (see below). Because [ATP] stays fixed during recovery (Kushmerick and Paul, 1976 *a*; cf. also Piiper and Spiller, 1970), the total suprabasal ATP production during recovery must just match the total suprabasal utilization of ATP during this time. This allows Eq. 8.1 to be deduced from Eq. 8. As stated in Eq. 8.2, the suprabasal ATP consumption during recovery can be written as the sum of two terms. In the first place, it must be at least as great as $\Delta[CP]_0$; during recovery, [CP] returns to its resting level (Dydynska and Wilkie, 1966; Janke et al., 1970; Kushmerick and Paul, 1976 *a*), and CP formation is known to occur only via creatine phosphokinase, at the expense of an equimolar amount of ATP. Second, any suprabasal hydrolysis of ATP would also constitute a fraction of the total suprabasal ATP utilization during recovery. It is assumed in

Eq. 8.2 that the amount of suprabasal ATP consumption by reactions other than these two can be neglected. However, this assumption is not essential to the main conclusion: if more terms were present in the numerator of the right-hand side of Eq. 8.2, it would still be true that $\Delta[CP]_0/\Delta[O_2]$ can closely match the $P:O_2$ ratio only if the total suprabasal ATP hydrolysis during recovery, as well as each additional term, is negligible in comparison with $\Delta[CP]_0$.

Expected Value of $\Delta[CP]_0/\Delta[O_2]$

In the calculation of the expected value for $\Delta[CP]_0/\Delta[O_2]$ in the absence of suprabasal ATP hydrolysis during recovery (6.1–6.5), it was assumed that suprabasal production of ATP occurs solely via oxidative metabolism (cf. Eq. 8). To the extent that other pathways for ATP production are operative, the expected value of $\Delta[CP]_0/\Delta[O_2]$ will be proportionally lower. Apparently, only

TABLE II
RELATIONSHIP BETWEEN $\Delta[CP]_0$ AND $\Delta[O_2]$

| | Tetanus duration | | | |
| | s | | | |
	0.2	0.5	1.0	Pooled
Chemical experiments*				
$\Delta[CP]_0$, $\mu mol/g$	1.44±0.25	2.98±0.29	4.31±0.24	
	(11)	(9)	(15)	
$P_{max} \cdot l_0/[C_T]$, $kg \cdot m/mol$	76.5±2.0	88.3±3.0	81.7±2.55	
	(11)	(9)	(15)	
Oxygen experiments*				
$\Delta[O_2]$, $\mu mol/g$	0.214±0.021	0.436±0.039	0.698±0.028	
	(12)	(7)	(8)	
$P_{max} \cdot l_0/[C_T]$, $kg \cdot m/mol$	70.9±2.4	80.0±1.4	78.7±2.4	
	(10)	(6)	(5)	
Ratios‡				
$\Delta[CP]_0/\Delta[O_2]$, $\mu mol/\mu mol$	6.72±(1.36)	6.83±(0.90)	6.18±(0.43)	6.58±(0.55)
	(14.5)	(14.0)	(21.0)	(25.5)
$\dfrac{\Delta[CP]_0/P_{max} \cdot l_0/[C_T])}{\Delta[O_2]/(P_{max} \cdot l_0/[C_T])}$, $\mu mol/\mu mol$	5.90±(1.21)	6.06±(1.06)	5.80±(0.49)	5.91±(0.54)
	(16.9)	(12.5)	(9.9)	(33.2)

Normalization of $\Delta[CP]_0$ and $\Delta[O_2]$ by $P_{max} \cdot l_0/[C_T]$ was based on the individual values of these parameters.

* All values are ± SEM. Number in parentheses is number of measurements.

‡ All values are ± SEM calculated from Eqs. 5–8. Number in parentheses is number of degrees of freedom calculated from Eqs. 3 and 8.

three such pathways need be considered: the creatine phosphokinase (CPK) and adenylate kinase reactions, and "anaerobic" glycolysis. During recovery, the CPK reaction is a net sink for ATP, rather than a source. ATP production via adenylate kinase is negligible even during tetanic contractions by anaerobic muscles at 20°C (Canfield and Marechal, 1973) and 0°C (Curtin and Woledge, 1975), and is presumably also negligible during aerobic recovery. In experiments similar to those reported here, but at 0°C, Kushmerick and Paul (1976 a) found that ATP synthesis coupled to the formation of lactate during recovery was about 5% as great as that coupled to O_2 consumption. If this is true at 20°C as well, the predicted value for $\Delta[CP]_0/\Delta[O_2]$ in the absence of suprabasal ATP hydrolysis would drop to 5.8–6.2.

Comparison with Previous Results

Kushmerick and Paul (1976 *b*), using an experimental design essentially the same as that of the present paper, but a different method for measuring $\Delta[O_2]$, found mean values of 3.5–4.4 for $\Delta[CP]_0/\Delta[O_2]$ in the sartorius of *R. pipiens* at 0°C. These values are significantly different from thsse reported here ($P <$ 0.001). This discrepancy appears not to be a temperature effect. Kushmerick (1977) has alluded to unpublished work performed with the same methodology at 20°C, for which $\Delta[CP]_0/\Delta[O_2]$ was 3.6, and DeFuria (1977) has since described similar experiments for which the mean values of $\Delta[CP]_0/\Delta[O_2]$ were 4.9, 4.4, and 4.1 for tetani of 1, 3, and 5 s, respectively. For the 1-s contractions, the value of $\Delta[CP]_0$ was very similar to that reported here; the discrepancy in $\Delta[CP]_0/\Delta[O_2]$ is thus almost entirely due to a discrepancy in $\Delta[O_2]$. In the
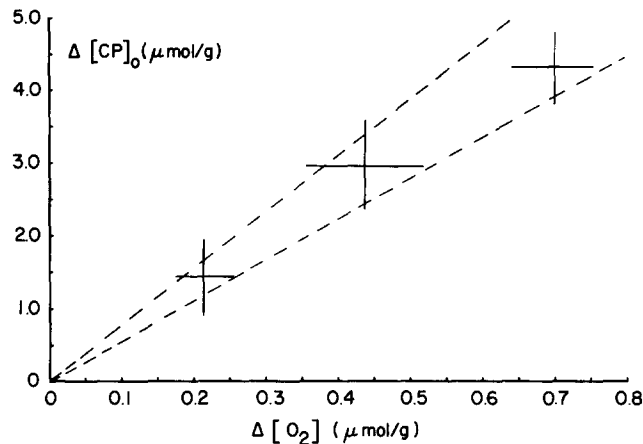

FIGURE 4. The relationship between $\Delta[CP]_0$ and $\Delta[O_2]$ for isometric tetani of 0.2, 0.5, and 1.0 s. The bars denote ± 1 SEM. The 95% confidence limits for ($\Delta[CP]_0/\Delta[O_2]$), 5.45 and 7.71, are shown by the dashed lines.

present paper, $\Delta[O_2]$ has been computed as the integral during recovery of the suprabasal rate of O_2 consumption, $\Delta Q_{O_2}(t)$. An error in $\Delta[O_2]$ could result from an error in either the nondimensionalized time-course of ΔQ_{O_2}, or in its absolute value. For example, if ΔQ_{O_2} reaches its peak by the end of a tetanus, as suggested by Kushmerick and Paul (1976 *a*) for 0°C, and not after 30–90 s, as reported here (cf. Fig. 1), the calculated integral of ΔQ_{O_2} could underestimate $\Delta[O_2]$. However, as discussed previously (Mahler, 1978 *b*), a delayed peak in $\Delta O_{O_2}(t)$ after a tetanus appears to be a real phenomenon, and, in general, the kinetics of ΔQ_{O_2} measured with the present method appear to be in satisfactory agreement with previously published evidence. On the other hand, even if the general kinetics of ΔO_{O_2} reported here are correct, a systematic error in $\Delta[O_2]$ might still exist: $\Delta Q_{O_2}(t)$ is calculated from the time-course of P_{O_2} at the muscle surface (Mahler, 1978 *b* and footnote 3); in this calculation, the transfer function $H(s)$ is a function of α, the volume-averaged solubility of O_2 in the muscle, and ΔQ_{O_2} is proportional to α (cf. Eqs. 2 and 3 of Mahler, 1978 *b*); inasmuch as this

[3] Mahler, M. A comparison of methods for computing time-variant system input from output and transfer function. Submitted for publication.

parameter has not been measured directly, an assumed value has been used, which, if inaccurate, would lead to a proportional error in ΔQ_{O_2}. Again, however, it does not seem likely that this type of error is large; as discussed in the first paper of this series (Mahler, 1978 a), the assumed values of α appear consistent with indirectly measured ones.

Kushmerick and Paul (1976 a,b) measured muscle oxygen consumption as the removal of O_2 from a Ringer-filled 4-ml chamber, using a macro-O_2-electrode to monitor the O_2 content of the chamber. It is not clear that this method is sufficiently sensitive to measure absolute values of $\Delta[O_2]$ of the magnitude reported here, 0.2–1.0 μl (i.e., 3–17 μl O_2/g in a 60-mg muscle; cf. Fig. 3). To illustrate, a 4-ml chamber equilibrated with, say, 50% O_2 at 20°C contains ~60 μl O_2. A suprabasal O_2 consumption by the muscle of 1 μl or less thus amounts to roughly 1% of the amount present in the chamber; moreover, this consumption takes 20–40 min, so its measurement calls for an extremely stable measure of chamber $[O_2]$. By comparison, micro-O_2-electrodes can show a drift of ~1%/ h in a constant P_{O_2} (Mahler, 1978 b), of the same order as the average value of ΔQ_{O_2} in the above example. The recording system used by Kushmerick and Paul (1976 a) may have been even less stable: when the chamber was filled with fresh Ringer solution, as when a muscle was mounted, the O_2 electrode current took 14 h to stabilize, during which time it had fallen to 4% of its initial level; it is not clear to what extent this fall was due to O_2 consumption by the electrode and (or) O_2 loss from the chamber, and to what extent it was due to recording system drift per se. It seems justified to mention in this context that the kinetics of ΔQ_{O_2} reported by Kushmerick and Paul (1976 a) differ in several important respects from those described by other workers (cf. Mahler, 1978 b for discussion).

The only other direct evidence on this topic has been provided by work on the in situ dog gastrocnemius. Piiper et al. (1968) measured $\Delta Q_{O_2}(t)$ during the transition from rest to a steady state after the onset of a train of brief contractions, and also measured, via muscle biopsies, the changes in [ATP] and [CP] between rest and the steady state of work. Since [ATP] showed no net change, and since ATP production linked to lactate formation was small under these conditions (DiPrampero et al., 1970), it follows that Δ[CP] closely approximated the amount of ATP not produced by oxidative metabolism during its transient, and that the ratio of Δ[CP] and the corresponding "oxygen deficit" should be analogous to the ratio $\Delta[CP]_0/\Delta[O_2]$ measured in the present experiments. On the assumption that the train of contractions caused a step increase in the rate of ATP splitting, the oxygen deficit was calculated as the integral of $[(\Delta Q_{O_2})_{ss} - \Delta Q_{O_2}(t)]$. When Piiper et al. (1968) plotted [CP] against this oxygen deficit, a roughly linear relationship was obtained that led to a value of 6.9 for $\Delta[CP]/\Delta[O_2]$, consistent with the results reported here. Piiper and Spiller (1970) monitored $\Delta Q_{O_2}(t)$ during recovery from steady-state work in the dog gastrocnemius, and measured [CP] during the steady state and at the end of recovery. $\Delta Q_{O_2}(t)$ had a fast component of the form e^{-kt}, and a more prolonged, roughly linear component. The ratio of Δ[CP] and the area under the fast component of ΔQ_{O_2} was 5.6; when the entire recovery oxygen consump-

tion was used, the ratio was 3.4. If the slow component of ΔQ_{O_2} reflects suprabasal ATP utilization during recovery, the ratio of $\Delta[CP]$ and the area under the exponential component is analogous to the ratio $\Delta[CP]_0/\Delta[O_2]$ in the present experiments. The results of Piiper and Spiller thus appear to be consistent with those of this work as well as those of Kushmerick and Paul (1976 b).

Suprabasal Hydrolysis of ATP during Recovery

The kinetics of $\Delta Q_{O_2}(t)$ reported here and previously (Mahler, 1978 b) suggest that the basal rates of oxygen consumption, and by implication, of ATP hydrolysis, at the end of recovery from a brief isometric tetanus can be slightly different from those before contraction. The values of $\Delta[CP]_0/\Delta[O_2]$ reported here imply that, with the exception of these small changes, the suprabasal splitting of ATP during recovery was negligible in comparison with that during a tetanus, at least for tetani of up to 1.0 s. At the 95% confidence level, however, the range of values of $\Delta[CP]_0/\Delta[O_2]$ consistent with the present results extends as low as 5.5, or if the parameters are normalized for tension, 4.8. Thus, assuming a $P:O_2$ ratio of 6.1–6.5, a suprabasal splitting of ATP during recovery that is 10–25% of that during a tetanus cannot be ruled out at this level of probability. Nevertheless, the agreement of the mean values of $\Delta[CP]_0/\Delta[O_2]$ for the three tetanus durations suggests that they do closely approximate the true value.

A postcontractile hydrolysis of CP (and thus, by implication, of ATP) might be masked by the resynthesis of CP via recovery metabolism. In muscles whose oxidative and glycolytic metabolism has been blocked, however, an appreciable postcontractile CP hydrolysis would result in a measurable decrease in [CP]. For these conditions, Lundsgaard (1934, summarized in Kalckar, 1969) found no change in [CP] during the 3 min immediately after a 4-s isometric tetanus at 20°C, or a 10-s tetanus at 10°C, results consistent with those reported here. At 0–2°C, Lundsgaard (1934) did detect a relatively large postcontractile CP breakdown after a 25-s tetanus. However, similar experiments with modern rapid-freezing techniques have failed to confirm this result. Marechal and Mommaerts (1963) found no significant CP breakdown in the frog sartorius during the 1,000 s after tetani of various durations at 0°C, and similar negative results have been reported by Gilbert et al. (1971; Table VI: $P > 0.05$ for $\Delta[CP]$, $P > 0.4$ for $\Delta[Pi]$) and Kushmerick and Paul (1976 b).

DeFuria and Kushmerick (1977) used an indirect method to test for suprabasal postcontractile ATP hydrolysis at 20°C by frog sartorii which were anoxic, but in which glycolysis was functional. They concluded that appreciable suprabasal hydrolysis of ATP had occurred during a recovery period of ~4 h after a brief tetanus. However, extrapolation of this result to the case of an adequately oxygenated muscle is problematical. In fact, some results of DeFuria and Kushmerick suggest that the prolonged anoxia to which these muscles were subjected may have caused fundamental changes in their metabolism. It was assumed by the authors that the basal ATP utilization by their preparations was entirely underwritten by the basal lactate production. If so, the basal metabolism

was in two respects very different from that in the aerobic state. First, it is apparent that the basal rate of lactate production increased continually during these experiments, at a relatively rapid rate, with overall changes as great as sixfold. In contrast, in well-oxygenated muscles, the basal rate of ATP utilization, as reflected by the basal Q_{O_2}, remains approximately constant for many hours at 20°C (Fenn, 1927; Fenn and Latchford, 1932). Second, the basal rate of ATP utilization by these muscles appears to have been only about 15–20% of that in well-oxygenated preparations. An average value of ~0.022 μmol ATP/g wet wt·min can be calculated for these experiments. In contrast, a rate of ~0.13 μmol/g·min can be calculated to occur in an oxygenated frog sartorius at 20°C; this figure is based on a resting Q_{O_2} of 0.5 μl O_2/g·min (Hill, 1966; Mahler, 1978 a), and a P:O_2 ratio of 6. For anaerobic frog muscle, the nuclear magnetic resonance (NMR) data of Burt et al. (1977) and Burt[4] can be used to deduce a resting rate of ATP utilization, based on the rate of decrease in [CP], of 0.15 μmol/g·min at 28°C; assuming a Q_{10} of 2 (cf. Discussion in Mahler, 1978 b), this is equivalent to 0.09 μmol/g·min at 20°C, reasonably close to the calculated value for aerobic muscle. It therefore appears that in the preparations of DeFuria and Kushmerick (1977), basal metabolism may have been severely depressed. Recovery metabolism may also have been somewhat different from that in an oxygenated muscle, and as the results of the present paper suggest, the large postcontractile ATP hydrolysis at 20°C observed by DeFuria and Kushmerick may occur only under anoxic conditions.

Implications for Control of Muscle Oxygen Consumption

Isometric tetani were intended to produce impulse-like changes in the rate of ATP hydrolysis by the frog sartorius. As calculated in the preceding paragraph, the resting rate of ATP hydrolysis in this muscle at 20°C is about 0.13 μmol/g·min. During tetani of 0.2–1.0 s, the data of Table I show that this rate increased by a factor of 2,000–3,000. On the time scale of oxidative recovery at 20°C, contractions of a second or less can be considered instantaneous, so the time-course of the rate of ATP hydrolysis in the vicinity of time $t = 0$ satisfactorily approximates that of an impulse. The present results imply that the total suprabasal ATP hydrolysis during recovery is small in comparison with that during a tetanus; it follows that the rate of suprabasal ATP hydrolysis must be negligible during this time, and thus that its time-course for $t > 0^+$ also approximates that of an impulse function. This establishes that $\Delta Q_{O_2}(t)$, the time-course of the rate of suprabasal oxygen consumption after a tetanus, can be considered the response to an impulse in the rate of suprabasal ATP hydrolysis. As shown in this and the preceding paper in this series, this response is well described by the two-component exponential expression (Eq. 7). Some preliminary implications of these results have been discussed previously (Mahler, 1978 b).

In Vivo P:O_2 Ratio

A fundamental assumption made in this study is that the P:O_2 in the intact frog sartorius matches that determined by classical in vitro work. Evidence consistent

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[4] Burt, C. T. Personal communication.

with this proposition has been reported by Skoog et al. (1978), who found a P:O ratio of 2.8 ± 0.1 in frog skeletal muscle mitochondria at 28°C. The results reported here are also consistent with this assumption, but in the present context, $\Delta[CP]_0/\Delta[O_2]$ cannot properly be considered an estimate of the P:O_2 ratio. As stated in Eq. 8.2, $\Delta[CP]_0/\Delta[O_2]$ will approximate the P:O_2 ratio only if suprabasal hydrolysis of ATP during recovery is small in comparison with $\Delta[CP]_0$. In this paper, the latter condition was established only by assuming that the P:O_2 ratio in vivo was in fact 6.1–6.5. Additional evidence is required to break this circle. It is certain, however, that in general, the P:O_2 ratio must be at least as great as $\Delta[CP]_0/\Delta[O_2]$ (cf. Eq. 8.2). The present results thus imply that the pooled mean for $\Delta[CP]_0/\Delta[O_2]$, 6.58 ± 0.55 ($n = 26$), sets an experimental lower bound on the P:O_2 ratio in the intact frog sartorius at 20°C.

Implications for Muscle Energy Balance

During brief isometric contractions by an unpoisoned frog sartorius at 0°C or 20°C, the only net reaction that has been detected chemically is the hydrolysis of creatine phosphate:

$$CP \rightarrow Cr + P \tag{10}$$

(Woledge, 1971; Curtin and Woledge, 1975). According to Woledge (1972), the enthalpy charge accompanying Eq. 10 and its associated buffer reactions in vivo is ∼ −34kJ/mol CP (−8.1 kcal/mol). The energy liberation during an isometric tetanus at 0°C is in fact approximately proportional to the CP breakdown, but with a proportionality constant averaging about −46kJ/mol (−11 kcal/mol) (Woledge, 1971; Homsher et al., 1975) or even higher (Gilbert et al., 1971). At 20°C, the data of Canfield et al. (1973) yield similar values: for tetani of 2 and 4 s, during which changes in metabolites other than CP could be neglected, ratios of −40.7 and −45.4 kJ/mol (−9.7 and −10.9 kcal/mol) can be calculated. This discrepancy implies that unknown chemical reactions occurring during contraction account for at least 25% of the observed energy liberation, and raises the possibility that such reactions might be at least partially driving contraction or related processes. A driving reaction might be expected a priori to be either more fundamental than ATP hydrolysis, but reversed by it, or not linked with ATP hydrolysis, and thus occurring in parallel with it; the latter possibility, however, seems remote (Atkinson, 1971). For tetani of up to 1 s at 20°C, the results of the present paper imply that if a driving reaction exists that is sequentially reversed by ATP splitting, its reversal is essentially complete by the end of relaxation: since $\Delta[CP]_0/\Delta[O_2]$ was in agreement with the expected range of the P:O_2 ratio, it follows that the ATP produced by oxidative recovery metabolism was essentially all used to restore [CP] to its resting level, and that the amount used for the reversal of reactions other than CP breakdown must have been relatively quite small.

The present results also make possible an indirect estimate, similar to that of Woledge (1971), of the value of $h_I/\Delta[CP]$ during a tetanus, where h_I denotes the initial heat. This estimate is based on the general postulates of muscle energy balance for a contraction/recovery cycle. It is normally assumed that if during a

contraction a number of net reactions have occurred, h_I will be given by $\sum n_i \, \Delta H_i$, where n_i denotes the extent of the ith reaction, and ΔH_i is its molar enthalpy. It is an experimental finding that h_I is approximately proportional to n_{CP}. The proportionality constant can be considered an "apparent in vivo enthalpy for CP hydrolysis," and designated ΔH_{app}. Thus, $h_I \simeq n_{CP} \cdot \Delta H_{app}$. It is assumed that during recovery, the initial net reactions are all reversed at the expense of oxidative metabolism. The total recovery heat is thus given by:

$$h_R = -\Sigma n_i \, \Delta H_i + C \cdot n_{O_2}, \tag{11}$$

where C has the value 4.94 kcal/liter O_2, or equivalently, 497 kJ/mol (119 kcal/ mol) at 20°C. If p denotes the ratio n_{CP}/n_{O_2}, and R, the ratio h_R/h_I, it follows that:

$$R \simeq \frac{-(n_{CP} \cdot \Delta H_{app}) + C(n_{CP}/p)}{n_{CP} \cdot \Delta H_{app}}, \tag{12}$$

and thus that:

$$\Delta H_{app} \simeq \frac{C}{p(R + 1)}. \tag{13}$$

The results of this paper give p as ~6.5 for short tetani at 20°C; the value of R is in the range 1.1–1.3 in the frog sartorius at 20°C (Hill, 1966; Godfraind-deBecker, 1973). Eq. 13 thus yields a value of 33.2–36.4 kJ/mol (8.0–8.7 kcal/ mol) for ΔH_{app} under these conditions. This deduced value of h_I/n_{CP} is in satisfactory agreement with the in vitro value of ΔH_{CP} calculated by Woledge (1972), and suggests that for tetani of up to 1 s in sartorius of *R. pipiens* at 20°C, by the end of relaxation an essentially complete energy balance is given by $E = n_{CP} \cdot \Delta H_{CP}$, with $\Delta H_{CP} \simeq 8.5$ kcal/mol. On the other hand, if direct energy balance study on this muscle at 20°C shows otherwise, as suggested by the results of Canfield et al. (1973) with *R. temporaria*, then provided the assumptions underlying Eq. 13 are in fact valid, the results presented above will place constraints on the enthalpy of unidentified initial exothermic processes reversed after relaxation. For example, in order to resolve the discrepancy between the in vitro value for ΔH_{CP} of -34 kJ/mol and a value for $\Delta h/\Delta[CP]_0$ of -46 kJ/mol, it would be necessary that the reversal of a reaction which liberated -12 kJ/mol $\Delta[CP]_0$ during contraction be coupled to a hydrolysis of ATP which is probably on the order of 0.05 mol ATP/mol $\Delta[CP]_0$. If the postulated reversal is stoichiometrically coupled to ATP hydrolysis, the unknown process must have an extremely high enthalpy, on the order of -240 kJ/mol (57 kcal/mol).

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