Kinetics of Oxygen Consumption after a Single Isometric Tetanus of Frog Sartorius Muscle at 20°C

MICHAEL MAHLER
From the Department of Physiology, School of Medicine, University of California at Los Angeles, Los Angeles, California 90024. Dr. Mahler’s present address is the Department of Pharmacology, School of Medicine, University of Southern California, Los Angeles, California 90033.

ABSTRACT The time-course of the rate of oxygen consumption (\(\dot{Q}_{O_2}\)) has been measured in the excised frog sartorius muscle after single isometric tetani of 0.1–1.0 s at 20°C. To measure \(\Delta\dot{Q}_{O_2}(t)\), the change in \(\dot{Q}_{O_2}\) from its basal level, a novel method was devised, based on the validity in this tissue of the one-dimensional diffusion equation for oxygen, established in the preceding paper. After a tetanus, \(\Delta\dot{Q}_{O_2}\) reached a peak within 45–90 s, then declined exponentially, and could be well fit by \(\Delta\dot{Q}_{O_2}(t) = \dot{Q}_0 + \dot{Q}_1(e^{-\tau_2 t} - e^{-\tau_1 t})\), \(\tau_2 = 1/k_2\), which characterized the rise of \(\Delta\dot{Q}_{O_2}\), was a decreasing function of tetanus duration (range: from 1.1 ± 0.28 min \([n = 5]\) for a 0.1-s tetanus, to 0.34 ± 0.05 min \([n = 8]\) for a 1.0-sec tetanus). \(\tau_1 = 1/k_1\), which characterized the decline of \(\Delta\dot{Q}_{O_2}\), was not dependent on tetanus duration, with mean 3.68 ± 0.24 min \((n = 46)\). A forthcoming paper in this series shows that these kinetics of \(\Delta\dot{Q}_{O_2}\) are the responses to impulse-like changes in the rate of ATP hydrolysis. The variation of \(\tau_2\) with tetanus duration thus indicates the involvement of a nonlinear process in the coupling of \(O_2\) consumption to ATP hydrolysis. However, the monoexponential decline of \(\Delta\dot{Q}_{O_2}(t)\), with time constant independent of tetanus duration, suggests that during this phase, the coupling is rate-limited by a single reaction with apparent first order kinetics.

INTRODUCTION

Two fundamental tenets of muscle energetics are that the free energy for cell function and maintenance is entirely provided by the hydrolysis of adenosine triphosphate (ATP), and that the primary source of ATP is the oxidation of substrates by molecular oxygen. A coupling between the hydrolysis of ATP and its resynthesis via oxidative metabolism thus appears essential for normal muscle function, and the elucidation of the mechanisms by which this occurs is a central problem in the study of metabolic control in muscle (Chance et al., 1962; Jöbsis, 1964; Jacobus and Lehninger, 1973; Owen and Wilson, 1974; Saks et al., 1974, 1976). These efforts are handicapped, however, by the absence of a general quantitative description of the dynamics of this coupling as it exists in an intact muscle.

From the point of view of systems analysis, it is natural to consider the events which link oxygen consumption to ATP hydrolysis as a system, for which the
input is the rate of ATP hydrolysis, and the output is the rate of oxygen consumption (QO₂), both considered as functions of time. From measurements of the kinetics of QO₂ elicited by impulse changes in the rate of ATP hydrolysis, it is possible to decide whether the system is linear, and if it is, to formulate a single system equation, valid at all times, which relates the QO₂ to the rate of ATP hydrolysis (Milsum, 1966; Riggs, 1970). In the experiments described in this paper, it was intended that stimulation of the excised sartorius muscle of *Rana pipiens* for 0.1–1.0 s at 20°C would produce a time-course of change in its rate of ATP hydrolysis which, on the time scale of oxidative recovery metabolism, would satisfactorily approximate an impulse. During the stimulation, the muscle performed a maximal isometric contraction. The experiments were designed to quantify the time-course of change in QO₂ from its basal level (∆QO₂) after the tetanus, and it was thus intended that these kinetics would represent an impulse response of the system.

The kinetics of recovery oxygen consumption in an isolated muscle at 20°C do not appear to have been previously reported. The method used here to measure ∆QO₂(t) takes advantage of the fact that QO₂ and intramuscular PO₂ in the excised frog sartorius are linked by the one-dimensional diffusion equation for oxygen (Eq. 1 below; Gore and Whalen, 1968; Mahler, 1978b). The time-course of PO₂ at a closed surface of a muscle was measured before, during, and after an isometric tetanus; in terms of Eq. 1, this was P(0, t). Given P(0, t), techniques of systems theory were used to solve the diffusion equation for ∆QO₂(t).

**MATERIALS AND METHODS**

**Measurement of PO₂ at the Muscle Surface**

The technique used to measure the time-course of PO₂ at a closed muscle surface during and after an isometric contraction was identical to that described in the preceding paper (Mahler, 1978b). For the present experiments, a pair of stimulating electrodes and a strain gauge were incorporated into the muscle chamber. The stimulating electrodes were situated on the chamber floor and lay perpendicular to long axis of a muscle, between the pelvic bone and the oxygen electrode. The strain gauge was attached to a clamp which held the pelvic bone. The experimental protocol before the stimulation of a muscle was essentially the same as that for the method I experiments described previously. A drained muscle was mounted in the chamber at its in vivo length, with the oxygen electrode recessed, and the chamber was then immersed in the water bath. The chamber gas composition was usually 75.2% O₂, 3.0% CO₂, and 21.8% N₂, but in a few experiments was 95% O₂, 5% CO₂. A few minutes after the muscle temperature had reached 20°C, the oxygen electrode was brought into contact with the lower surface of the muscle. The subsequent time-course of the PO₂ at the muscle surface was generally similar to that in the method I experiments described in the preceding paper, but was not formally analyzed. In the present context, the purpose of this period was to ensure that before the muscle was stimulated, the surface PO₂, and by implication, the QO₂ and the intramuscular PO₂ profile, had become constant. If the PO₂ trace did not eventually become level, the muscle was discarded; this occurred in about 10% of experiments. If the PO₂ trace became level, the muscle was stimulated for 0.1–2.0 s at a just supramaximal voltage, with stimuli of duration 0.6 ms and frequency 70 Hz. In all cases, the contraction was isometric.

During the contraction, and in some cases for several seconds thereafter, the electrode
current rose above its precontraction level. Usually, this rise had the form of a spike of small amplitude, which appeared to be caused at least in part by pressure exerted on the electrode during the tetanus, and possibly in part by electrical phenomena. After the spike, the electrode current was in most cases at its original level, and then began a gradual decline due to the change in PO₂ at the muscle surface (cf. Fig. 1). Occasionally, the current immediately after the spike was marginally higher or lower than its precontraction level, and this was attributed to a slight repositioning of the muscle as a result of the tetanus, with an attendant small change in the muscle thickness over the cathode. In cases when the electrode current was still elevated above its precontraction level after ~10 s from the beginning of the stimulation, records were discarded. As explained in the following section, the first reading from acceptable records was usually not taken until 24 s after the contraction. Records obtained after an isometric tetanus were analyzed only if they eventually returned to a steady level, indicating that the QO₂ had become constant. After the last suitable record of P(0, t) had been obtained, the oxygen electrode was withdrawn from the muscle surface, and again exposed to the chamber gas; this made it possible to measure the drift of the recording system during the entire experimental period. This averaged ~1% per h. The drift was assumed to have occurred at a constant rate, and the PO₂ records were corrected accordingly.

Calculation of Q(t) from P(0, t)

For these calculations, it was assumed that the intramuscular PO₂ profile was related to the QO₂ by the one-dimensional Fick diffusion equation:

$$D \frac{\partial^2 P}{\partial x^2}(x, t) - \alpha \frac{\partial P}{\partial t}(x, t) = Q(t), \quad (1)$$

where P is the partial pressure of oxygen (PO₂), Q is the QO₂, x is the distance perpendicular to the muscle surface, t is time, α is the solubility of oxygen in muscle, and D is the diffusion coefficient for oxygen. This equation tacitly assumes that QO₂ is uniform throughout the muscle, and thus varies only with time. If so, Eq. 1 implies that for each time-course of change by the surface PO₂ from its initial steady-state value, denoted ΔP(0, t), there is a unique ΔQ(t).

The idea of calculating the kinetics of QO₂ in an isolated tissue from the kinetics of PO₂ at its surface, via the one-dimensional diffusion equation for O₂, appears to have originated with the work of Connelly et al. (1953) on isolated nerve. However, the mathematical techniques used by these authors were accurate only in special cases. For the present paper, a numerical method derived from the theory of linear systems has been used, which allows the calculation of ΔQ(t) from an arbitrary ΔP(0, t). The diffusion equation has the form of a system differential equation, with Q(t) as the input, and P(x, t) the output. As shown in Appendix 1, for a muscle oxygenated only from one surface, and there by a constant PO₂, for the case x = 0 the transfer function for this system has the form:

$$H(s) = \frac{\text{sech}(\sqrt{s/D}) - 1}{\alpha s}, \quad (2)$$

where t is the thickness of the muscle above the platinum cathode, and s is a dummy complex variable. ΔQ(t) can be expressed in terms of ΔP(0, t) by the equation:

$$\Delta Q(t) = \mathcal{F}^{-1} \left\{ \mathcal{F} \left[ \Delta P(0, t) \right] \right\} (t), \quad (3)$$

where $\mathcal{F}$ and $\mathcal{F}^{-1}$ denote the direct and inverse Fourier transforms. $\omega$ is a dummy
complex frequency, and \( j = \sqrt{-1} \). The derivation of Eq. 3 is also given in Appendix I. In the present case, \( \Delta P(0, t) \) is not known in closed form, but only as a series of points. In practice, therefore, the exact Fourier transforms in Eq. 3 were approximated by their discrete forms, as computed by a Fast Fourier Transform (FFT) routine (Brigham, 1974). The resulting \( \Delta Q(t) \) was thus also given as a series of points, with the same time base as \( \Delta P(0, t) \). The values used for \( D \) and \( \alpha \) in Eq. 2 were \( 1.34 \times 10^{-9} \text{ cm}^2/\text{s} \) and \( 0.0307 \mu \text{L O}_2/\text{g} \cdot \text{mm Hg} \), respectively (Mahler, 1978b).

To determine the errors inherent in the use of the discrete Fourier transform rather than its exact form, and as a general test of the computational method, an experiment was simulated analytically. The muscle was assumed to be initially in steady state, and \( Q_{O_2} \) was then allowed to change from its basal level with the time course \( \Delta Q(t) = e^{-t} - e^{-2t} \). The diffusion equation was then solved to yield an exact expression for the corresponding change in \( P(0, t) \); cf. Appendix II. As required by the FFT routine, this curve was then sampled at a fixed time interval, and using this data, \( \Delta Q(t) \) was calculated via the method outlined above. With as few as 64 output points, the calculated values matched the known, exact values with 1% accuracy; the total amount of extra oxygen consumed, \( \int_0^\infty \Delta Q(t) \, dt \), was approximated to about 2% accuracy by numerically integrating the calculated points with the trapezoidal rule. For higher sampling rates, the calculated and exact values of \( \Delta Q(t) \) could be made virtually identical. This method can be generally applied to recover the input to any stable linear system for which the output and transfer function are known, and is considerably more accurate than the "unit response" method traditionally used for the correction of myothermal records (Hill, 1966, Chapter 13). The transform method is discussed in greater detail in a separate paper (Mahler, 1978a).

In practice, because of the large amounts of computer storage necessary to process records of >128 points, the experimental records of \( P(0, t) \) were usually sampled at 24-s intervals, and either 64 or 128 points were used in the FFT routines. Curve fitting to the calculated points for \( \Delta Q_{O_2}(t) \) was done with a nonlinear least squares method (Brown and Dennis, 1970).

With a few exceptions, all experiments were done during the months of December and January.

**RESULTS**

*Kinetics of \( Q_{O_2} \) after an Isometric Tetanus*

Fig. 1 shows a typical recording of the \( P_{O_2} \) at the lower surface of a muscle after an isometric tetanus. In every case, these records displayed an initial decline along an S-shaped path, with inflection point at \( \sim 1 \) min, to a minimal level reached after \( \sim 3-5 \) min, followed by a gradual rise back to a steady level, again with an S-shaped time-course, with the final level reached after 20-50 min. On the average, for contractions of 0.1-0.4 s, this final \( P_{O_2} \) matched the precontraction value; for longer tetani, the final \( P_{O_2} \) tended to be slightly lower than the initial level. The longer the duration of the tetanus, the larger was the transient in the surface \( P_{O_2} \). The relative time course of \( P_{O_2} \) showed little variation with the tetanus duration in a given muscle, at least for contractions of 0.1-1.0 s, although from one muscle to the next, the time spans involved could vary considerably.

It should be emphasized that according to the diffusion equation, the intramuscular \( P_{O_2} \) was at its lowest at the surface where it was being measured,
and where it was typically at least 200 mm Hg (e.g., cf. Fig. 1), well in excess of the reported "critical Po2" for this muscle (Hill, 1948; Gore and Whalen, 1968). It seems safe to infer that the QO2 was never limited by O2 delivery.

Fig. 2 shows examples of the time-course of the suprabasal rate of oxygen consumption, ΔQ(t), calculated from the records of surface Po2; for the sake of clarity, the discrete values of ΔQ(t) have been connected by continuous lines. The oscillations evident in these records, while no doubt due in part to both variation in the surface Po2 and to errors in sampling it, can also be attributed in part to the use of discrete rather than exact Fourier transforms in the calculation of ΔQ(t) via Eq. 3 (Brigham, 1974).

In almost all cases, it was apparent that the descending limb of ΔQ(t) could be well approximated by a single exponential; accordingly, the best-fitting curve of the form \( a + be^{-kt} \) was calculated for each record. Although the goodness of fit was difficult to quantify, it was nevertheless evident from visual inspection that in almost all cases, the fits could be considered quite good in about 90% of all cases. Curves a and b of Fig. 2 are examples of average fits, chosen as much to illustrate typical deviations from an exponential time-course, as a strict adherence to it: in curve a, the oscillations in the latter part of the curve are unusually large; in curve b, after ΔQ(t) has dropped to about 10% of its peak value, it remains slightly but consistently higher than the best-fitting exponential for about 10 min; as discussed below, this tendency was accentuated for tetani of longer than 1.0 s. The fit shown in Fig. 2c was among the best observed.

\[ r = \sqrt{\frac{\sum_{i} (\text{error})^2}{\sum_{i} (\Delta Q_i)^2}} \]

which approximates the average relative error, did not appear to be a particularly useful index of the goodness of fit. In cases for which the oscillations around the fitted curve were relatively large, but still quite uniform, so that the fit would be subjectively judged very good, the values of r were typically relatively large; in comparison, smaller values of r were often obtained when the deviations were smaller, but less uniformly distributed about the fitted curves, so that the fits appeared by inspection to be considerably worse. The average value for r was about 0.15; for records a, b, and c of Fig. 2, the values of r are 0.147, 0.107, and 0.098, respectively.
FIGURE 2. Typical records of the calculated time-course of \( \Delta Q_o_2 \) after isometric tetani of 0.1–1.0 s at 20°C. Tetanus durations (a) 0.2 s, (b) 0.8 s, and (c) 0.5 s. Curve b is derived from the \( P_o_2 \) record shown in Fig. 1. Smooth curves: best-fitting curves of the form \( a + b e^{-\lambda t} \).
It was determined in early experiments that, after a 2-s tetanus, the Po$_2$ at the closed surface of the muscles sometimes fell to zero and remained there for several minutes; this occurred in muscles 1.0-1.2 mm thick, despite a Po$_2$ of about 540 mm Hg at the upper surface. These experiments proved incidentally useful, by providing further evidence that, for a Po$_2$ of zero, the electrode current was the same whether the external medium was a muscle or a test gas, an assumption that was made routinely in the calibration of the electrode currents recorded from muscle (cf. Methods in Mahler, 1978b). For the main body of experiments, tetani of 1 s or less were used, to ensure that the muscles were well oxygenated. However, several experiments were done in which muscles were adequately oxygenated after tetani of 1.2-2.0 s. Fig. 3 shows the results of one such experiment. In general, the time-course of $\Delta QO_2$ was at first similar to that described above for shorter tetani; once it had fallen to ~20% of its peak value, however, the subsequent decline to a steady value was markedly slower than for the shorter tetani, so that the entire descending limb of $\Delta QO_2$ was poorly fit by a single exponential.

Fig. 4 illustrates the dependence on tetanus duration of the time constant of the descending limb of $\Delta QO_2(t)$; for reasons made clear below, this time constant will be designated $\tau_1$. The values plotted represent all experiments with tetani of 0.1-1.0 s ($n = 46$). Linear regression showed no dependence of $\tau_1$ on tetanus duration ($r = -0.041$, $P > 0.7$); this conclusion is clouded, however, by the scatter in the values of $\tau_1$ (range, 1.9-8.0 min). An alternative approach was to consider only cases in which two or more experiments had been done with a single muscle, and to compare, for all possible pairs of such experiments, the value of $\tau_1$ for the longer tetanus of the pair to that for the shorter. The results of these comparisons are shown graphically in Fig. 5. When the values of $\tau_1$ were plotted against each other pairwise, the points clustered along the line of identity; for the 59 possible pairs, based on 45 experiments in 14 muscles, the
mean value of $(\tau_{\text{longer tetanus}}/\tau_{\text{shorter tetanus}})$ was $0.999 \pm 0.022$. Clearly, any dependence of $\tau_1$ on the tetanus duration must be slight over the range 0.1-1.0 s. The mean value of $\tau_1$ for all experiments was $3.68 \pm 0.24$ min ($n = 46$); inasmuch as the distribution was somewhat skewed, it is also of interest that the median value was 3.09 min.

The final value of $\Delta Q_{O_2}$ after a tetanus, denoted $(\Delta Q_{O_2})_{0}$, represents the change in the basal level of $Q_{O_2}$ from its precontraction value. For tetani of 0.1-0.4 s, $(\Delta Q_{O_2})_{0}$ was negligible (pooled mean = $0.0012 \pm 0.0048 \mu l/g \cdot min$, $n = 19$). For tetani of 0.5 s and longer, the average value of $(\Delta Q_{O_2})_{0}$ ranged from 0.02 to 0.11 $\mu l/g \cdot min$; in comparison, the resting $Q_{O_2}$ at 20°C was previously observed to be about 0.5 $\mu l/g \cdot min$ (Mahler, 1978b). $(\Delta Q_{O_2})_{0}$ was quite variable for tetani of 0.5 s and longer: it was sometimes near zero, even for tetani of 2.0 s (cf. Fig. 3), and was significantly different from zero only for 1.0 s tetani. However, a small increase in the basal $Q_{O_2}$ does appear to have occurred in some experiments.

Except for the fact that $\Delta Q_{O_2}$ reaches its peak value only after 45-90 s, its general time-course as reported here is well described as the response of a first order system to an impulse input. An exact first order response would have the form:

$$\Delta Q_{O_2}(t) = \Delta Q_{O_2}(0) \cdot e^{-t/\tau_1},$$

and this time-course was in fact proposed by Kushmerick and Paul (1976) to describe their results at 0°C. This raises the question of whether the present experiments can distinguish with certainty between the observed kinetics and those of Eq. 4. A related but more fundamental question is whether the calculated 45-90 s rise in $\Delta Q_{O_2}$ is a methodological artifact. With regard to the second question, two factors can be identified that might cause the recorded
time-course of \( P_{O_2} \) to change more slowly than that actually occurring at the lower surface of the muscle; this would in turn cause the calculated \( \Delta Q_{O_2}(t) \) to lag behind the true curve. The first factor is a lag in the response of the system used to record \( P_{O_2} \); the second is the existence of a layer of connective tissue and Ringer fluid between the muscle fibers and the oxygen electrode. Their effects were deduced by making appropriate modifications in the equations by which \( \Delta Q_{O_2}(t) \) is linked to the observed \( P(0, t) \), calculating the new values for \( \Delta Q_{O_2}(t) \) from a sample experimental record of \( P(0, t) \), and then comparing these new values to the original calculated time-course of \( \Delta Q_{O_2} \). The response time of the recording system was incorporated into Eq. 3 as an additional first order system, with a time constant of 2.0 s (Mahler, 1978b), and as expected, the resulting changes in \( \Delta Q_{O_2}(t) \) were negligible.

To estimate the effect on the calculated \( \Delta Q_{O_2} \) of the layer of connective tissue and Ringer solution lying between the muscle fibers and the oxygen electrode, it was assumed that the \( P_{O_2} \) profile within this layer was determined by the one-dimensional diffusion equation (Eq. 1), that its rate of oxygen consumption was zero, and that \( D \) and \( \alpha \) had the same values there as in water. The existence of the layer necessitates a change in the boundary condition for \( P_{O_2} \) at the lower surface of the muscle; the derivation of the modified system transfer function relating \( \Delta Q_{O_2}(t) \) to the observed \( P(0, t) \) is given in Appendix IV of Mahler (1976).

It was assumed that 25 \( \mu \)m was a generous upper limit for the thickness of the nonconsuming layer. According to Hill (1949), the excised frog sartorius has at its "outer" surface, which was originally next to the skin, a layer of connective tissue with average thickness about 6 \( \mu \)m, whereas at the "inner" surface, no such layer is evident. In the present experiments, it was the inner surface which

![Figure 5](image-url)
was in contact with the oxygen electrode, so the nonconsuming layer was probably composed simply of Ringer solution. Hill (1949) estimated the thickness of the layer of Ringer solution between a drained muscle and a thermopile to be about 1 or 2 μm. In the present context, it seems realistic to accept this only as a lower limit; using the fact that a muscle weighs ~6% more when drained than when blotted, it can be calculated that if in a drained muscle this extra weight is due to a layer of Ringer solution spread uniformly over the entire periphery, then the thickness of the layer will be about 20 μm for muscles of the size used in the present work. Accordingly, in calculating the effect on ΔQO₂(t) of the nonconsuming layer, it was assumed that the layer was no more than 25 μm thick. Even when this figure was used, the calculated time-course of ΔQO₂ was only slightly changed: during the first 1–2 min, individual values were generally increased by 2–10%, but the time at which the peak in ΔQO₂ occurred was the same; in the latter portion of the curve, the values were in general slightly smaller; the area under the curve and the time constant τ₁ were smaller by only a few percent. Because these effects were small, and no measurements were made of the thickness of the nonconsuming layer, corrections of this type were not incorporated into the results.

It remains to be considered whether the methodology used here makes it possible to rule out a monoexponential time-course for ΔQO₂ during the entire recovery period. This question was answered by assuming that ΔQO₂(t) did in fact have the form proposed in Eq. 4, calculating the corresponding time-course of PO₂ at the muscle surface predicted by the diffusion equation, and comparing this record to those observed experimentally (for details, cf. Appendix II). The results are shown graphically in Fig. 6. ΔQO₂(t) can, for the purpose of these calculations, be well approximated by the function \( e^{-k₁t} - e^{-k₂t} \), where \( k₁ \) and \( k₂ \) have the values 0.29 min⁻¹ and 2.0 min⁻¹, corresponding to time constants of 3.5 min and 30 s, respectively. These observed kinetics are shown in the inset of Fig. 6 as curve a. The corresponding monoexponential time-course, \( e^{-k₁t} \), appears as curve b. Curve c is the time-course of ΔPO₂ at the muscle surface predicted by Eq. 1 when ΔQO₂(t) is specified by curve a; it closely matches the experimental records (cf. Fig. 1). Curve d shows, on the same scale, the time-course of ΔPO₂ which would occur if ΔQO₂(t) was given by b rather than a.

Given exact values from curve c, the transform method can recover the input \( e^{-k₁t} - e^{-k₂t} \) with a high degree of accuracy (cf. Methods, and Mahler, 1978a); given points from curve d, its input \( e^{-k₁t} \) can also be approximated with good accuracy.² In practice, points from experimental records were read by eye; this was presumably done with small random errors, but these can be shown to have a negligible effect on the accuracy of the transform method. It follows that if the micro-O₂-electrode and recording system were sufficiently stable, responsive, and sensitive that a PO₂ transient described by curve d would not be recorded as curve c, it will then be demonstrated that the method used here was adequate to distinguish a QO₂ transient of the form \( e^{-k₁t} - e^{-k₂t} \) from one of the form \( e^{-k₁t} \). This apparatus was stable to within 1% per h, and had a time

² Mahler, M. Unpublished observation.
constant of about 2 s (cf. Mahler, 1978b). Although no specific experiments were done to determine the limits of its sensitivity, its excellent linearity implies that changes in \( P_0 \) on the order of a few mm Hg can be measured, and previous workers have routinely used similar instruments for this purpose (e.g. Connelly et al. 1953; Gore and Whalen, 1968; Kawashiro et al., 1975; Kushmerick and Paul, 1976). In contrast, Figs. 1 and 6 illustrate that under the conditions of the present experiments, the observed records describe large changes in electrode current, with maximum deflections on the order of 50%, and occur gradually over many minutes; moreover, on a realistic scale, a curve of form \( d \) would differ from one of form \( c \) by as much as 30-40 mm Hg. It follows that according to the methodology employed in this paper, it can be concluded that after a tetanus of 0.1-1.0 s, \( \Delta Q_{02}(t) \) does not have the form \( Q_{ae} - k t \).

A somewhat more subtle question is whether any constants \( C \) and \( k \) can be found for which a monoexponential \( \Delta Q_{02}(t) \) given by \( Ce^{-kt} \) can produce a time-course of \( \Delta P_0 \) which will match, within experimental error, the observed kinetics given by curve \( c \). Given the validity of Eq. 1, this possibility can also be ruled out. For a monoexponential \( \Delta Q_{02}(t) \), \( \Delta P_0 \) will always have a cusp at \( t = 0 \), and a monotonically increasing slope during the initial phase of recovery (cf. curve \( d \)); in contrast, the experimental records invariably followed an S-shaped path during this time, with a pronounced “shoulder” at the start (cf. Fig. 1 and curve \( c \)). This initial discrepancy could be lessened, and the trough points in \( \Delta P_0 \) made to coincide, only by choosing \( k \) considerably smaller than \( k_1 \), with appropriate scale factor \( C \); this resulted in a wide divergence between the ascending limbs of the predicted and observed curves of \( \Delta P_0 \). These results argue that the initial 45-90-s rise in \( \Delta Q_{02} \) calculated by the present method is a real phenomenon, and it thus appears justified to incorporate these early kinetics into the quantification of \( \Delta Q_{02}(t) \).
The entire time-course of $\Delta Q_{O_2}$ could be approximated by the expression:

$$Q_0 + Q_1(e^{-k_1 t} - e^{-k_2 t});$$  (5)

cf. Fig. 7. $Q_0$ is the final value of Eq. 5 after a tetanus, and $Q_0 + Q_1$ is the value obtained by extrapolating its monoexponential descending limb back to $t = 0$. For the determination of the best-fitting values of $k_2$ and $Q_1$, $\Delta Q_{O_2}(t)$ was fit by Eq. 5 with $k_1$ and $Q_0$ held constant at the values previously determined by fitting the declining limb of $\Delta Q_{O_2}(t)$ only. Reasonable fits could sometimes be obtained with other functions: e.g., a linear rise followed by a monoexponential fall (cf. Fig. 2c); however, most records showed a gradual rounding in the vicinity of the peak value which was consistent with Eq. 5. Nevertheless, the values of $\Delta Q_{O_2}(t)$ during its rise and early fall were generally not as well fit by Eq. 5 as was its descending limb, during which Eq. 5 was essentially monoexponential. It seems appropriate to consider the rate constant $k_2$ simply as a phenomenological parameter which, via Eq. 5, provides a good first approximation to the early kinetics of $\Delta Q_{O_2}(t)$, but which does not necessarily deserve a strict mechanistic interpretation. Unlike $\tau_1$, $\tau_2 (= 1/k_2)$ was dependent on the tetanus duration (cf. Fig. 8). When values of $\tau_2$ for different tetanus durations in a single muscle were compared pairwise, $\tau_2$ for the longer tetanus of the pair was smaller than that for the shorter tetanus in 53 of 59 cases ($P < 0.001$ if $\tau_2$ were independent of duration). The parameter $Q_1$ increased in curvilinear fashion with the tetanus duration.

For tetani of 1.2–2.0 s, and occasionally for shorter tetani, $\Delta Q_{O_2}(t)$ could not be well fit by Eq. 5: components of the form $e^{-k_1 t}$ and $e^{-k_2 t}$ were still apparent, but it was necessary to also include in the description of $\Delta Q_{O_2}(t)$ an approximately linear component substantially slower than $e^{-k_2 t}$ (cf. Fig. 3).

As shown in Fig. 9, the maximal value of $\Delta Q_{O_2}$ after a tetanus was related to the tetanus duration by a slightly curvilinear function. After a 2-s tetanus, it was about 6.5 $\mu l/g \cdot min$, or about 13 times the resting $Q_{O_2}$. For a given tetanus
duration, this peak value of $\Delta QO_2$ was linearly related to the rate constant ($k_1$) which characterized its subsequent fall. Inasmuch as both parameters might be expected to depend on the number of mitochondria in a muscle, this result suggests a high level of internal consistency in these measurements of $\Delta QO_2(t)$.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure8}
\caption{The relationship between the duration of an isometric tetanus and the time constant $\tau_2$ of $QO_2(t)$ after the tetanus.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure9}
\caption{The relationship between the duration of an isometric tetanus and the peak value of $\Delta QO_2(t)$ after the tetanus.}
\end{figure}

**DISCUSSION**

*Spatial Dependence of $QO_2$ in the Frog Sartorius*

The method presented here for the measurement of $\Delta QO_2$ is based on the validity of the diffusion Eq. 1, which assumes that the rate of oxygen consumption is the same throughout the muscle. Several lines of evidence support this assumption. Histochemical investigation showed that cross sections of the sartorius of *R. pipiens* stain uniformly for myosin ATPase and succinate dehydrogenase, indicating that the sites of ATP hydrolysis and oxygen con-
assumption are uniformly distributed. Single fiber cross sections typically cut 40-100 mitochondria, and the average area "served" by a mitochondrion is roughly 20 μm². The validity of Eq. 1 in the resting sartorius (Mahler, 1978b) indicates that the resting ΔO₂ is uniform throughout the muscle. Finally, results consistent with the uniformity of muscle oxygen consumption were provided by experiments designed to test the effect of the placement of the oxygen electrode on the measured ΔQ(t) for a tetanus of 0.2 s. In the first of a pair of experiments, a muscle was mounted in the usual way, with the platinum cathode ~8 mm from the pelvic bone, and approximately equidistant from the lateral edges of the muscle (cf. Fig. 1 of Mahler, 1978b). For the second experiment, the positions of the ends of the muscle were exchanged, so that the cathode now lay 8 mm from the distal end, and 20-25 mm from the pelvic bone. On average, Q₀, Q₁, τᵢ, τₛ, and the total oxygen consumption ∫ ΔQ(t) agreed to within 10% for the two electrode placements (n = 6).

Comparison with Previous Work

TIME-COURSE OF ΔO₂. The results presented here show that after a single brief isometric tetanus of 20°C, the time-course of ΔO₂ in the excised frog sartorius quickly becomes monoexponential. These kinetics are consistent with virtually all previous observations in this field, made on skeletal muscles of the frog at 0°C, for which τ = 10-20 min (Hill, 1940a; Kushmerick, and Paul, 1976), and at 12°C (τ = 10 min, Baskin and Gaffin, 1965); the dog (τ = 24 s at 36°C, Piiper et al., 1968); and man (τ = 45 s; for review cf. Berg, 1947, and Casaburi et al., 1977). The kinetics of oxygen consumption in frog skeletal muscle at 20°C have apparently not been previously described; however, Godfraind-deBecker (1972, 1973) reported that after isometric tetani of 0.5-1.5 s by excised toad and frog sartorii at 20°C, the rate of heat production became monoexponential after 2-3 min, with a time constant of 3.3-4.0 min, and that NADH fluorescence in the toad sartorius had essentially the same kinetics during this time span. Both of these processes can be expected to occur in parallel with ΔO₂ (Hill, 1940a, b; Jöbsis and Duffield, 1967). The results of Godfraind-deBecker (1972, 1973) thus seem quantitatively consistent with those reported here.

According to the present results, the time constant of the descending limb of ΔO₂(t) after a tetanus is independent of the tetanus duration over the range 0.1-1.0 s. This conclusion is also consistent with virtually all previous results, except those of Kushmerick and Paul (1976), who reported that after single tetani in the sartorius of R. pipiens at 0°C, the exponential time constant for ΔO₂ increased markedly with the tetanus duration over the range 1-30 s. In contrast, Hill (1940a) concluded on the basis of similar experiments with R. temporaria that the time constant for ΔO₂ was invariant for tetani of up to 20 s. Moreover, the rate of aerobic recovery heat production, which according to Hill (1940a, b) parallels ΔO₂, has also been reported to have a time constant which does not vary with the tetanus duration, in excised sartorii of the frog at 0°C (Hill, 1940b) and the toad at 20°C (Godfraind-de Becker, 1973). Analogous results have been reported for canine and human skeletal muscle. Piiper et al.

3 Eisenberg, B., and A. Kuda. Unpublished observation.
(1968) used the Fick principle to approximate transient kinetics of oxygen uptake by the *in situ* dog gastrocnemius during series of tetani of fixed duration and rate; these series presumably caused approximately stepwise increases in the rate of ATP utilization by the muscle, in contrast to the impulse increase which presumably accompanies a single tetanus (cf. Introduction). The transients in oxygen uptake measured by Piiper et al. could be approximated by curves of the form \((1 - e^{-\tau t})\), with \(\tau\) independent of the steady state \(Q_o2\) over a wide range in the latter. In the context of a systems analysis of the link between ATP splitting and oxygen consumption, these results are consistent with the independence of \(\tau\) from tetanus duration reported by Hill (1940a) and in this paper. Moreover, numerous studies on the kinetics of oxygen uptake in man during work suggest that there is a wide range of conditions for which \(\Delta Q_o2\) has exponential kinetics in human skeletal muscle after a step change in work rate, with time constant again independent of step size (cf. Casaburi et al., 1977 for review).

According to the present results, \(\Delta Q_o2\) in the frog sartorius does not have exclusively monoexponential kinetics after a single tetanus at 20°C; an initial rapid component is present which results in a delayed rise to the peak value. The only strictly comparable published evidence appears to be that reported by Hill (1940a) and Kushmerick and Paul (1976) for the excised frog sartorius at 0°C. Hill's results were similar to those reported here, in that differentiation of his records of cumulative suprabasal oxygen consumption after a tetanus indicates that \(\Delta Q_o2(t)\) reached its peak value only after 2-3 min. In contrast, Kushmerick and Paul concluded that \(\Delta Q_o2(t)\) was at its peak by the end of a tetanus. Examination of their experimental records shows that suprabasal oxygen uptake was essentially zero for several minutes after a tetanus; because the rate of \(O_2\) consumption is the sum of the rate of uptake and the rate at which the muscle \(O_2\) store is decreasing, it follows that for \(\Delta Q_o2(t)\) to have been maximal by the end of a tetanus, the calculated rate of depletion of the \(O_2\) store of the muscle must have been relatively large during the early phase of recovery. This rate was calculated via the diffusion equation (Eq. 1), with the diffusion coefficient for oxygen in muscle at 0°C assumed to have the value \(2.75 \times 10^{-4}\) cm²/min (Hill, 1966). However, as reported in the preceding paper (Mahler, 1978b), although Eq. 1 does appear to be valid in the excised frog sartorius, \(D\) has the value \(4.94 (\pm 0.16) \times 10^{-4}\) cm²/min at 0°C, from which it follows that the actual changes in the \(O_2\) store of the muscle were only about half as large as those calculated by Kushmerick and Paul (1976), and that the initial phase of \(\Delta Q_o2(t)\) in their experiments may have been similar to that reported here and by Hill (1940a). A similar criticism in fact applies to the results of Hill (1940a) as well, and implies that the peak values of \(\Delta Q_o2\) in his experiments occurred somewhat later than is evident from his corrected records. Indirect measures of the early kinetics of \(\Delta Q_o2\) in amphibian skeletal muscle after single tetani also appear consistent with the results reported here. A delayed rise to a maximum has been reported for the NADH fluorescence change in the toad sartorius at 12°C (Jóbsis and Duffield, 1967) and 20°C (Godfrain and deBecker, 1972, 1973), and for the rate of aerobic recovery heat production in the frog sartorius at 0°C.
According to the results summarized in Fig. 9, the longer the duration of an isometric tetanus, the faster is the rise of $\Delta Q_{O_2}(t)$ to its peak value after the tetanus. Although no comparable analysis of the early kinetics of $\Delta Q_{O_2}(t)$ after a contraction has been published, similar behavior has been reported for the rate of aerobic heat production in the frog sartorius at 20°C (Hartree and Hill, 1922; Hill, 1966) and for NADH fluorescence in the toad sartorius at 12°C (Jöbsis and Duffield, 1967).

SENSITIVITY Oxygen uptake by an excised muscle has previously been measured directly, as the amount of $O_2$ removed from a well-stirred chamber (for methods cf. Fenn, 1927; Gemmill, 1936; Hill, 1940a; Kushmerick and Paul, 1976). A potential difficulty with such methods is that the rate at which oxygen disappears from the chamber is often quite small in comparison with the amount actually present. For example, $O_2$-filled chambers typically contain roughly 5 ml $O_2$ (Fenn, 1927; Gemmill, 1936; Hill, 1940a; Baskin and Gaffin, 1964); even a Ringer-filled chamber (Kushmerick and Paul, 1976) of 5 ml, if bubbled with 95% $O_2$ at 20°C, would contain 140 $\mu$l $O_2$. In comparison, according to the present results, the total suprabasal oxygen consumption by a 60-mg muscle after a 1.0 s tetanus is only $\sim$1 $\mu$l, or for a 0.1-s tetanus, 0.2 $\mu$l; moreover, these volumes are consumed only over a period of 20-40 min. With the method described in this paper, the kinetics of muscle oxygen consumption are not measured directly, but deduced from the kinetics of $PO_2$ at a muscle surface. Although this technique undoubtedly entails a more complicated set of assumptions than previous methods, it appears to offer considerably greater sensitivity. For an appropriate choice of the chamber gas, the change in surface $PO_2$ after a tetanus can be made to constitute a large, easily measured fraction of the initial value. For example, with a chamber gas containing 5-10% $O_2$, it is possible to measure $\Delta Q_{O_2}(t)$ in the frog sartorius after a single isometric twitch at 0°C.2

Implications for Control of $Q_{O_2}$

The overall aim of this and the accompanying papers (Mahler, 1978 b, c) has been to provide data which would make it possible to quantify the dynamic coupling between the rates of ATP hydrolysis and $O_2$ consumption in well oxygenated muscle cells. If a system is defined by specifying these rates as its input and output, respectively, the problem at hand becomes one of system identification. Evidence presented in a forthcoming paper in this series indicates that during and after a single isometric tetanus of 1 s or less at 20°C, the time-course of the suprabasal rate of ATP hydrolysis in the sartorius of R. pipiens can be well described as an impulse, and it follows that in the present context, the kinetics of $\Delta Q_{O_2}$ reported here for tetani of 1 s or less are those of impulse responses. The application of these results to the determination of general equations linking the rates of ATP hydrolysis and $O_2$ consumption in this muscle is intended to be the topic of a separate paper. However, some fundamental conclusions can be noted here. First, the system is nonlinear. If it were linear, then for tetani of increasing duration, which produce impulse-like inputs of
increasing area, the time constants $\tau_1$ and $\tau_2$ of $\Delta Q_{O_2}(t)$ would remain fixed. The variation of $\tau_2$ with tetanus duration (cf. Fig. 8) thus indicates the involvement of a nonlinear process. Second, the monoexponential decline of $\Delta Q_{O_2}(t)$ from its peak value after a tetanus, with time constant ($\tau_1$) independent of tetanus duration (cf. Figs. 4 and 5), suggests that during this period, the sequence of events linking ATP hydrolysis and $O_2$ consumption is rate-limited by a single reaction with apparent first order kinetics. An essentially similar hypothesis was advanced by Hill (1940a) to explain his results at 0°C.

**ADDENDUM**

For the calculation of $\Delta Q(t)$, the transfer function $H(s)$ which links $\Delta P(0, t)$ and $\Delta Q(t)$ has been evaluated via Eq. 2, using an average value of $D$ based on previous experiments, and individually measured values of $l$. During the review of this paper, it was pointed out that if an experiment of the type described here is accompanied by one done with the muscle in the resting state, by either of methods I or II described in the preceding paper, a simpler representation is possible for $H(s)$. In the method I and II experiments, the time-course of $P_{O_2}$ at the muscle surface becomes monoexponential, with rate constant $k = \pi^2 D / 4l^2$. $H(s)$ can thus be expressed as:

$$H(s) = \frac{\text{sech}(1.571 \sqrt{s/k}) - 1}{\alpha s}.$$  

Eq. 6 appears preferable to Eq. 2 for two reasons. First, it is potentially more accurate, because it involves one less measured parameter; moreover, $k$ can usually be measured with excellent accuracy, especially with method II (cf. Fig. 5 of Mahler, 1978b). Second, if Eq. 6 is used, the experimental procedure for measuring $\Delta Q(t)$ can be simplified considerably, because the muscle thickness $l$ need not be measured (cf. Methods).

**APPENDIX I**

**Derivation of Eqs. 2 and 3**

The one-dimensional diffusion equation,

$$D \frac{\partial^2 P}{\partial x^2}(x, t) - \frac{\partial P}{\partial t}(x, t) = Q(t),$$

has the form of a system differential equation, for which $Q(t)$ is the input, and $P(x, t)$ is the output. For the conditions of the present experiments, the initial and boundary conditions on $P(x, t)$ are:

$$P(x, 0) = P_a - \frac{Q_a}{2D\alpha}(x^2 - \bar{x}^2); \quad (1.2a)$$

$$P(l, t) = P_a; \quad (1.3a)$$

and

$$\frac{\partial P}{\partial x}(0, t) = 0; \quad (1.4a)$$
where \( P_0 \) denotes the \( P_{O_2} \) of the chamber gas, \( Q_0 \) is the basal rate of oxygen consumption, and \( l \) is the muscle thickness. For a change in \( Q(t) \) from \( Q_0 \), denoted \( \Delta Q(t) \), the corresponding forced output is \( \Delta P(x, t) \), where:

\[
\Delta Q(t) = Q(t) - Q_0, \tag{1.5a}
\]

and

\[
\Delta P(x, t) = P(x, t) - P(x, 0). \tag{1.6a}
\]

The purpose of this Appendix is to derive the transfer function of this system for the case \( x = 0 \), i.e., to evaluate the function:

\[
H(s) = \frac{\mathcal{L}[\Delta P(0, 0)](s)}{\mathcal{L}[\Delta Q(t)](s)}. \tag{1.7a}
\]

This analysis can be simplified by setting \( Q_0 = 0 = P_0 \). These conditions do not alter the form of \( H(s) \), but allow Eqs. 1.2a, 1.3a, 1.5a, and 1.6a to be rewritten:

\[
P(x, 0) = 0; \tag{1.8a}
\]
\[
P(l, t) = 0; \tag{1.9a}
\]
\[
\Delta Q(t) = Q(t); \tag{1.10a}
\]

and

\[
\Delta P(x, t) = P(x, t). \tag{1.11a}
\]

It will be shown below that the explicit form of Eq. 1.7a is:

\[
H(s) = \frac{\text{sech}(l\sqrt{s/D}) - 1}{\alpha s}. \tag{1.12a}
\]

This equation is derived below by first taking the Laplace transform of Eq. 1.1a with respect to \( t \), then solving the resulting ordinary differential equation in \( x \).

The Laplace transform with respect to \( t \) of Eq. 1.1a is:

\[
0^2 \psi + \alpha^2 \phi + \beta \phi(x, s) = \mathcal{L}[Q(t)](s), \tag{1.13a}
\]

where \( \mathcal{L}[P(x,t)](s) \) and \( \mathcal{L}[Q(t)](s) \). Eq. 1.13a is an ordinary differential equation in \( x \), which for the sake of simplification can be written as:

\[
\frac{d^2 y}{dx^2} + k^2 y = \beta, \tag{1.14a}
\]

where

\[
y(x) = \mathcal{L}[P(x, s)]; \tag{1.15a}
\]
\[
k^2 = s/D; \tag{1.16a}
\]

and

\[
\beta = \mathcal{L}[Q(t)]/D\alpha. \tag{1.17a}
\]

One way to solve Eq. 1.14a is to take its Laplace transform with respect to \( x \), which yields:

\[
\alpha^2 \cdot \tilde{y}(u) - k^2 \tilde{y}(u) = \frac{\beta}{\mu} + \mu \cdot y(0) + y'(0), \tag{1.18a}
\]
where \( \bar{y}(u) \) denotes \( \mathcal{L}[y(x)](u) \). The boundary condition Eq. 1.4a on \( P(x, t) \) can be used to show that \( y'(0) = 0 \), as follows:

\[
\bar{y}(x) = \mathcal{L}_d[P(x, t)](s);
\]

\[
\Rightarrow y'(x) = \frac{dy}{dx}(x) = \frac{d}{dx} \{\mathcal{L}[P(x, t)](s)\} = \mathcal{L} \left[ \frac{\partial P}{\partial x} (x, t) \right](s);
\]

\[
\Rightarrow y'(0) = \mathcal{L} \left[ \frac{\partial P}{\partial x} (0, t) \right](s) = \mathcal{L}[0](s) = 0.
\]

It thus follows from Eq. 1.18a that:

\[
\bar{y}(u) = \frac{\beta + \mu^2 y(0)}{(\mu^2 - k^2)\mu}
\]

\[
= \frac{A}{\mu + k} + \frac{B}{\mu - k} + \frac{C}{\mu}.
\]

Evaluating the partial fractions in Eq. 1.23a gives:

\[
A = \frac{\beta}{2k^2} + \frac{y(0)}{2};
\]

\[
B = A;
\]

\[
C = -\beta/k^2.
\]

The solution of Eq 1.14a is thus:

\[
y(x) = A(e^{-\alpha x} + e^{\beta x}) + C +
\]

\[
= \left[ \frac{\beta}{k^2} + y(0) \right] \left( e^{-\alpha x} + e^{\beta x} \right) - \frac{\beta}{k^2}
\]

\[
= y(0) \cdot \cosh(kx) + \frac{\beta}{k^2} [\cosh(kx) - 1].
\]

Using Eqs. 1.16a and 1.17a,

\[
y(x) = y(0) \cdot \cosh(x/\sqrt{D}) + \frac{\hat{Q}(s)}{\alpha s} [\cosh(x/\sqrt{D}) - 1].
\]

The object of this derivation is to express \( \hat{P}(0, s) \), or \( y(0) \), in terms of \( \hat{Q}(s) \). This can be done by evaluating Eq. 1.30a at \( x = l \). From boundary condition Eq. 1.10a we have:

\[
y(l) = \hat{P}(l, s) = \mathcal{L}[P(l, t)](s) = 0.
\]

On the other hand, Eq. 1.30a implies that:

\[
y(l) = y(0) \cdot \cosh(l/\sqrt{D}) + \frac{\hat{Q}(s)}{\alpha s} [\cosh(l/\sqrt{D}) - 1].
\]

It follows that:

\[
y(0) = \frac{\hat{Q}(s)}{\alpha s} \left[ \frac{1 - \cosh(l/\sqrt{D})}{\cosh(l/\sqrt{D})} \right];
\]

\[
= \frac{\hat{Q}(s)}{\alpha s} [\text{sech}(l/\sqrt{D}) - 1].
\]
which is equivalent to Eq. 1.12a and to text Eq. 2.

Text Eq. 3 follows from Eq. 1.7a. For any function \( f(t) \), defined for \( 0 \leq t < \infty \), if one formally defines \( f(t) = 0 \) for \( \infty < t < 0 \), it follows from the definitions of the Laplace and Fourier transforms that:

\[
\mathcal{F}[f(t)](\omega) = \mathcal{F}[f(t)](j\omega).
\]

Therefore, it follows from Eqs. 1.7a and 1.35a that:

\[
H(j\omega) = \frac{\mathcal{F}[\Delta P(0, t)](\omega)}{\mathcal{F}[\Delta Q(t)](\omega)},
\]

which implies that:

\[
\mathcal{F}[\Delta Q(t)](\omega) = \frac{\mathcal{F}[\Delta P(0, t)](\omega)}{H(j\omega)}.
\]

Taking the inverse Fourier transform of Eq. 1.37a yields text Eq. 3.

**APPENDIX II**

Derivation of Exact Solution for \( \Delta P(0, t) \) when \( \Delta Q(t) \) has the forms \( e^{-kt} \) and \( (e^{-kt} - e^{-k't}) \)

The desired expression for \( \Delta P(0, t) \) can be obtained from the general formula:

\[
\Delta P(0, t) = \frac{-4}{\alpha \pi} \sum_{n=0}^{\infty} \frac{(-1)^n}{(2n+1)} \int_0^t \Delta Q(\tau) e^{-\frac{(2n+1)^2 \pi^2 D}{4D} \tau} d\tau.
\]  

(2.1a)

Eq. 2.1a can be derived from the convolution integral:

\[
\Delta P(0, t) = \int_0^t h(t-\tau) \Delta Q(\tau) d\tau,
\]

(2.2a)

where:

\[
h(t) = S^{-1}[H(s)](t),
\]

(2.3a)

and \( H(s) \) is the system transfer function given in Eq. 2 of the text (for details, cf. Appendix V of Mahler, 1976).

\[
H(s) = \frac{\text{sech}(t\sqrt{s/D}) - 1}{\alpha s},
\]

(2.4a)

and it can be shown that its inverse Laplace transform is:

\[
h(t) = \frac{-4}{\alpha \pi} \sum_{n=0}^{\infty} \frac{(-1)^n}{(2n+1)} e^{-\frac{(2n+1)^2 \pi^2 D}{4D} t}.
\]

(2.5a)

For \( \Delta Q(t) = e^{-kt} \), it follows directly from El. 2.1a that:

\[
\Delta P(0, t) = \frac{-4}{\alpha \pi} \left\{ e^{-kt} \sum_{n=0}^{\infty} \frac{(-1)^n}{(2n+1)(\alpha_n - k)} - \sum_{n=0}^{\infty} \frac{(-1)^n e^{-\alpha_n t}}{(2n+1)(\alpha_n - k)} \right\}.
\]

(2.6a)

where:

\[
\alpha_n = (2n + 1)^2 \pi^2 D/4l^2.
\]

(2.7a)
For $\Delta Q(t) = e^{-kt} - e^{-kt'}$, evaluating Eq. 2.1a gives:

$$
\Delta P(0, t) = -4 \sum_{\sigma} \frac{(-1)^n e^{-n\sigma t}}{\pi} \left( - \frac{1}{2n + 1}(\alpha_n - k_1) \right) - \sum_{\sigma} \frac{(-1)^n e^{-n\sigma t}}{(2n + 1)(\alpha_n - k_1)} - \sum_{\sigma} \frac{(-1)^{n+1} e^{-k_2t}}{(2n + 1)(\alpha_n - k_1)} + \sum_{\sigma} \frac{(-1)^{n+1} e^{-k_2t'}}{(2n + 1)(\alpha_n - k_1)}.
$$

(2.8a)

To obtain curves e and d in Fig. 6, computer programs were written to evaluate Eqs. 2.6a and 2.8a.

I would like to thank Ivan Whitehorn, Nick Ricchiuti, and Bernard Tai for technical help, Robert Eisenberg, Arthur Peskoff, and Richard Mathias for stimulating discussions on mathematical matters, Chris Clausen for invaluable advice on APL programming, and Earl Homsher and Charles Kean for general discussions.

This work was supported by training grant HL-05696 and Program Project grant HL-11351 from the U. S. Public Health Service.

Received for publication 18 January 1978.

REFERENCES

BASKIN, R. J., and S. GAFFIN. 1965. Oxygen consumption in frog sartorius muscle. I. The isometric twitch. J. Cell. Comp. Physiol. 65:19-26.

BERG, W. E. 1947. Individual differences in respiratory gas exchange during recovery from moderate exercise. Am. J. Physiol. 149:597-610.

BRIGHAM, E. O. 1974. The Fast Fourier Transform. Prentice-Hall, Inc., Englewood Cliffs, N. J. 91-109, 124-125, 132-137.

BROWN, K. M., and J. E. DENNIS, Jr. 1970. Derivative free analogues of the Levenberg-Marquardt and Gauss algorithms for nonlinear least squares approximation. IBM Philadelphia Scientific Center Technical Report No. 320-2994, August, 1970.

CASABURI, R., B. J. WHIPP, K. WASSERMAN, W. L. BEAVER, and S. N. KOYAL. 1977. Ventilatory and gas exchange dynamics in response to sinusoidal work. J. Appl. Physiol. 42:300-311.

CHANCE, B., G. MAURIELLO, and X. AUBERT. 1962. ADP arrival at muscle mitochondria following a twitch. In Muscle as a Tissue. K. Rodahl and S. M. Horvath, editors. McGraw-Hill Book Company, New York. 128-145.

CONNELLY, C. M., D. W. BRONK, and F. BRINK. 1953. A sensitive respirometer for the measurement of rapid changes in metabolism of oxygen. Rev. Sci. Instrum. 24:683-695.

FENN, W. O. 1927. The gas exchange of isolated muscles during stimulation and recovery. Am. J. Physiol. 83:309-322.

GEMMILL, C. L. 1936. The respiratory metabolism of stimulated frog's muscle. Am. J. Physiol. 115:371-375.

GODFRAIND-DEBECKER, A. 1972. Heat production and fluorescence changes of toad sartorius muscle during aerobic recovery after a short tetanus. J. Physiol. (Lond.). 223:719-734.

GODFRAIND-DEBECKER, A. 1973. La restauration post-tétanique du muscle strié thermogénèse et fluorescence. Vander, Louvain, Belgium. 55-58.

GORE, R. W., and W. J. WHALEN. 1968. Relations among tissue $P_{O_2}$, $Q_{O_2}$, and resting heat production of frog sartorius muscle. Am. J. Physiol. 214:277-286.

HARTREE, W., and A. V. HILL. 1922. The recovery heat production of muscle. J. Physiol. (Lond.). 56:367-381.
Hill, A. V. 1949. Myothermic methods. *Proc. R. Soc. Ser. B Biol. Sci.* **136**:228–241.

Hill, A. V. 1966. Trails and Trials in Physiology. The Williams & Wilkins Company, Baltimore. 189–207, 211, 304–330.

Hill, D. K. 1940a. The time course of the oxygen consumption of stimulated frog’s muscle. *J. Physiol. (Lond.)* **98**:207–227.

Hill, D. K. 1940b. The time course of evolution of oxidative recovery heat of frog’s muscle. *J. Physiol. (Lond.)* **98**:454–459.

Hill, D. K. 1948. Oxygen tension and the respiration of resting frog’s muscle. *J. Physiol. (Lond.)* **107**:479–495.

Jacobs, W. E., and A. L. Lehninger. 1973. Creatine kinase of rat heart mitochondria. Coupling of creatine phosphorylation to electron transport. *J. Biol. Chem.* **248**:4803–4810.

Jöbsis, F. F. 1964. Basic processes in cellular respiration. *Hand. Physiol.* 1(Sect. 3. Respiration) 63–124.

Jöbsis, F. F., and J. C. Duffield. 1967. Oxidative and glycolytic recovery metabolism in muscle. Fluorometric observations on their relative contributions. *J. Gen. Physiol.* **50**:1009–1047.

Kawashiro, T., W. Nusse, and P. Scheid. 1975. Determination of diffusivity of oxygen and carbon dioxide in respiring tissue: results in rat skeletal muscle. *Pfluegers Arch.* **359**:231–251.

Kushmerick, M. J., and R. J. Paul. 1976. Aerobic recovery metabolism following a single isometric tetanus in frog sartorius muscle at 0°C. *J. Physiol. (Lond.)* **254**:693–709.

Mahler, M. 1976. Initial creatine phosphate breakdown and kinetics of recovery oxygen consumption for single isometric tetani of the frog sartorius muscle at 20°C. Ph.D. Dissertation. University of California at Los Angeles.

Mahler, M. 1978a. A comparison of methods for computing time-variant input to a biological system when output and transfer function are known. Submitted for publication.

Mahler, M. 1978b. Diffusion and consumption of oxygen in the resting frog sartorius muscle. *J. Gen. Physiol.* **71**:533–557.

Mahler, M. 1978c. The relationship between initial creatine phosphate breakdown and recovery oxygen consumption for a single isometric tetanus of the frog sartorius muscle at 20°C. Submitted for publication.

Milsom, J. H. 1966. Biological Control Systems Analysis. McGraw Hill Book Company, New York.

Owen, C. S., and D. F. Wilson. 1974. Control of respiration by the mitochondrial phosphorylation state. *Arch. Biochem. Biophys.* **161**:581–591.

Piper, J., P. E. DiPrampero, and P. Cerretelli. 1968. Oxygen debt and high energy phosphates in gastrocnemius muscle of the dog. *Am. J. Physiol.* **215**:525–531.

Riggs, D. S. 1970. Control Theory and Physiological Feedback Mechanisms. The Williams & Wilkins Company, Baltimore. 91–112.

Saks, V. A., G. B. Chernousova, Iu. I. Voronkov, V. N. Smirnov, and E. I. Chazov. 1974. Study of energy transport mechanism in myocardial cells. *Circ. Res.* **34-35**(Suppl. 3):138–148.

Saks, V. A., N. V. Lipina, V. N. Smirnov, and E. I. Chazov. 1976. Studies of energy transport in heart cells. The functional coupling between mitochondrial creatine phosphokinase and ATP-ADP translocase: kinetic evidence. *Arch. Biochem. Biophys.* **173**:34–41.