

Reappraisal of Diffusion, Solubility, and Consumption of Oxygen in Frog Skeletal Muscle, with Applications to Muscle Energy Balance

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ABSTRACT Previously we tested the validity of the one-dimensional diffusion equation for O₂ in the excised frog sartorius muscle and used it to measure the diffusion coefficient (*D*) for O₂ in this muscle and the time course of its rate of O₂ consumption (*Q*_{O₂}) after a tetanus (Mahler, 1978, 1979, *J. Gen. Physiol.*, 71:533–557, 559–580, 73:159–174). A transverse section of the frog sartorius is in fact well fit by a hemi-ellipse with width divided by maximum thickness averaging 5.1 ± 0.2. Using the previous techniques with the two-dimensional diffusion equation and this hemi-elliptical boundary yields a value for *D* that is 30% smaller than reported previously; the revised values at 0, 10, and 22.8°C are 6.2, 7.9, and 10.8 × 10⁻⁶ cm²/s, respectively. After a tetanus at 20°C, *Q*_{O₂} rose quickly to a peak and then declined exponentially, with a time constant (*τ*) ~15% faster than that reported previously; *τ* averaged 2.1 min in *Rana temporaria* and 2.6 min in *Rana pipiens*. A technique was devised to measure the solubility (*α*) of O₂ in intact, respiring muscles, and yielded *α*(muscle)/*α*(H₂O) = 1.26 ± 0.04. With these modifications, the values for O₂ consumption obtained with the diffusion method were in agreement with those measured by the direct method of Kushmerick and Paul (1976, *J. Physiol. [Lond.]*, 254:693–709). Using results from both methods, at 20°C the ratio of phosphorylcreatine split during a tetanus to O₂ consumption during recovery ranged from 5.2 to 6.2 μmol/μmol, and postcontractile ATP hydrolysis was estimated to be 13.6 ± 4.1 (*n* = 3) nmol/μmol total creatine.

INTRODUCTION

In previous papers of this series (Mahler, 1978*a, b*, 1979), the time course of the rate of oxygen consumption (*Q*_{O₂}) by the isolated frog sartorius muscle was calculated from the time course of the partial pressure of oxygen (*P*_{O₂}) at the muscle surface by numerical solution of the one-dimensional diffusion equation for O₂. This equation is

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

where *y* is the distance perpendicular to the muscle surface, *t* is time, *P*(*y*, *t*) is

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the P_{O_2} , D and α are the diffusion coefficient and solubility of O_2 in the tissue, and $Q(t)$ is the Q_{O_2} . This procedure appeared to be justified on several counts. Gore and Whalen (1968) had shown that in a resting excised muscle lying on a flat surface impermeable to oxygen, the intramuscular P_{O_2} profile along a line perpendicular to the supporting surface had the same form as the steady state solution of Eq. 1, and Mahler (1978*a*) found that under similar conditions, when a step change was made in the P_{O_2} at the upper surface of the muscle, the time course of P_{O_2} at the closed, lower surface also had the form predicted by Eq. 1. A comparison with a variety of previously published results indicated that the general form of the time course of Q_{O_2} determined with this method was substantially correct (Mahler, 1978*b*); however, there was some reason to question the absolute accuracy of the method. For an isometric tetanus of the frog sartorius at 0°C, Kushmerick and Paul (1976*b*) had measured the drop in phosphorylcreatine ($\Delta\{PC\}_0$),¹ an indirect measure of the amount of ATP hydrolyzed, and also the total suprabasal oxygen consumption during recovery ($\Delta\{O_2\}$), measured directly as the amount of O_2 removed by the muscle from a closed chamber. (This method is referred to hereafter as the "direct method.") $\Delta\{PC\}_0/\Delta\{O_2\}$ was $\sim 4.3 \mu\text{mol}/\mu\text{mol}$ for a range of tetanus durations. Kushmerick (1977) and DeFuria (1977) reported similar results at 20°C. However, Mahler (1979) measured the same quantities at 20°C, with $\Delta\{O_2\}$ calculated as $\int \Delta Q_{O_2}(t) dt$, and found $\Delta\{PC\}_0/\Delta\{O_2\}$ to be $6.6 \pm 0.6 \mu\text{mol}/\mu\text{mol}$. This discrepancy had an added significance because of the implications of the value of $\Delta\{PC\}_0/\Delta\{O_2\}$ for the problem of muscle energy balance (Woledge, 1971; Kushmerick and Paul, 1976*b*; Homsher and Kean, 1978; Mahler, 1979). We therefore undertook a re-examination of the assumptions underlying the "diffusion method."

Implicit in the one-dimensional diffusion equation is the assumption that the tissue is adequately approximated by a plane sheet. A. V. Hill (1966), in his treatment of diffusion of oxygen through tissues, suggested that the frog sartorius be treated as an elliptical cylinder for which the diffusion equation must include two space variables. In rectangular coordinates, this equation is

$$D\alpha\left(\frac{\partial^2 P}{\partial x^2} + \frac{\partial^2 P}{\partial y^2}\right) - \alpha\frac{\partial P}{\partial t} = Q. \quad (2)$$

However, Hill considered only the steady state solution to Eq. 2. The mathematical methodology needed to provide non-steady state solutions under the conditions of the diffusion method has now been developed (Mahler, 1985*a*). In the work described here, the fit of the frog sartorius by a hemi-elliptical cylinder was quantified, and past (Mahler, 1978*a*, *b*, 1979) and present experiments were analyzed using this improved description of the tissue configuration. This resulted in a substantial correction in the value of D , the diffusion coefficient for O_2 , and smaller changes in the form of $Q_{O_2}(t)$.

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

The Q_{O_2} measured by the diffusion method is directly proportional to the value used for α , the solubility of O₂ in the tissue (Mahler, 1978a). This was a possible source of error, since this quantity had apparently never been measured in muscle; the value used in all calculations had been that estimated by A. V. Hill (1966), i.e., $\alpha(\text{muscle}) = 0.85\alpha(\text{H}_2\text{O})$. However, Campos Carles et al. (1975) measured the solubilities of seven inert gases in rat skeletal muscle and found that $\alpha(\text{muscle})/\alpha(\text{H}_2\text{O})$ ranged from 1.1 to 2.4. We developed a method to measure α for O₂ in the frog sartorius, which yielded $\alpha(\text{muscle})/\alpha(\text{H}_2\text{O}) = 1.26 \pm 0.04$ ($n = 9$), which is ~50% higher than Hill's value.

After the diffusion method had been modified according to the results described above, it was tested by two sets of experiments. To test the accuracy of the form of the time course of Q_{O_2} , we made use of the demonstration by D. K. Hill (1940a, b) that during the recovery period after a single tetanus of the frog sartorius at 0°C, the time course of oxygen consumption had virtually the same form as the time course of heat production. For a 0.5-s tetanus of the sartorius of *Rana temporaria* at 20°C, we compared the form of the time course of the Q_{O_2} as measured by the diffusion method with that of the rate of production of recovery heat and found them to be practically identical. To test the absolute accuracy of the diffusion method, we used it to measure three quantities, all in the sartorius of *Rana pipiens* at 20°C, for which highly reliable values could be furnished by the "direct method" of Kushmerick and Paul (1976a). In each case, there was now good agreement between the results of the two methods. This enabled revised estimates to be made of $\Delta\{\text{PC}\}_0/\Delta\{\text{O}_2\}$ for single tetani at 20°C and of the total postcontractile suprabasal hydrolysis of ATP.

MATERIALS AND METHODS

Quantification of Tissue Configuration

A frog sartorius muscle was placed in a plastic tray with dimensions $3 \times 5 \times 0.3$ cm, with its length held constant at the value measured in situ. A mold of the muscle was made by covering it with a quick-drying dental casting material (Jeltrate, type II, L.D. Caulk Co., Dentsply Int., Milford, DE), diluted three times. Once the mold had set, it was removed from the tray and sectioned transversely 8 cm from the pelvic bone, at the point at which muscle P_{O_2} was measured with the diffusion method (Mahler, 1978b). The sectioned face of the mold was photographed, its image was projected onto a digitizing surface (Bitpad One, Summagraphics, Fairfield, CT), and 20–50 points from the upper surface of the muscle were digitized. The lower surface of the muscle was defined to lie on the x axis, $\{y = 0\}$. A nonlinear least-squares curve-fitting routine was used to determine the hemi-ellipse that best fit the points. The fitting function $y = f(x)$ was defined by:

$$\frac{(x - x_0)^2}{m^2} + \frac{y^2}{l^2} = 1, \quad |x - x_0| \leq m, \quad (3.1)$$

$$y = 0, \quad |x - x_0| > m. \quad (3.2)$$

Eq. 3.1 specifies the upper half of the ellipse with center at $(x_0, 0)$, with major axis lying on $\{y = 0\}$, and semi-minor and semi-major axes of lengths l and m , respectively. With this definition alone, convergence of the curve-fitting routine was not guaranteed, since the fitting error was undefined for data points whose x coordinate lay outside the major

axis of the ellipse. Eq. 3.2 quantifies such errors by defining the fitting function for these values of x . Other procedures for fitting these points can be envisioned; however, the suitability of Eq. 3.2 was rendered moot by the fitting protocol finally adopted, in which poorly fit points from the outer edges of the section were neglected. When all points were weighted equally, the length of the semi-minor axis (l) of the best-fitting hemi-ellipse was typically 5–10% smaller than the maximum observed tissue thickness. As discussed in Mahler (1985a), for a given boundary condition and sink strength, the diffusant concentration at the center of a hemi-elliptical medium is primarily determined by the central portion of the medium, with the extreme end regions having little effect. Therefore, in the fitting procedure, l was held constant at the maximum tissue thickness. Moreover, if it was evident that the fit to the central points would be noticeably improved by neglecting a few subjectively chosen points at either end, this was done (see Fig. 2). Longitudinal variations in the muscle configuration were ignored; the error introduced by this assumption appears to be negligible (Mahler, 1978b).

Measurement of $\Delta Q_{O_2}(t)$ by the Diffusion Method

This method is identical to that of Mahler (1978b), except that the tissue configuration was assumed to be a hemi-elliptical cylinder rather than a sheet. The P_{O_2} profile within the tissue was assumed to be described by the two-dimensional diffusion equation for O_2 (Eq. 2). Given the time course of P_{O_2} at the tissue surface, the time course of Q_{O_2} was obtained by numerical solution of Eq. 2, using techniques of systems analysis. In the terminology of Eq. 2, for a given point (x_0, y_0) , the diffusion equation defines a linear system with input $Q(t)$ and output $P(x_0, y_0, t)$. Let $\Delta Q(t)$ and $\Delta P(x_0, y_0, t)$ denote changes in $Q(t)$ and $P(x_0, y_0, t)$ from their resting steady state values. $\Delta Q(t)$ can be calculated from $P(x_0, y_0, t)$ by the algorithm

$$\Delta Q(t) \approx \mathcal{F}_D^{-1} \left\{ \frac{\mathcal{F}_D[\Delta P(x_0, y_0, t)](\omega)}{H(x_0, y_0, j\omega)} \right\} (t), \quad (4)$$

where $H(x_0, y_0, s)$ is the system transfer function, \mathcal{F}_D and \mathcal{F}_D^{-1} denote the direct and inverse discrete Fourier transforms, and j denotes $\sqrt{-1}$. In Mahler (1985a), it is shown that this transfer function has the form

$$H(x_0, y_0, s) = (1/\alpha) \sum_{n=0}^{\infty} \sum_{r=1}^{\infty} C_{n,r} \frac{1}{s + k_{n,r}}, \quad (5.1)$$

where

$$C_{n,r} = B_{2n,r} C e_{2n}(\xi_0, q_{2n,r}) c e_{2n}(\eta_0, q_{2n,r}), \quad (5.2)$$

$$k_{n,r} = q_{2n,r} (4D/l^2) \sinh^2 \xi_b, \quad (5.3)$$

and $\{B_{2n,r}\}$ are constants whose form is given in Mahler (1985a). Eqs. 5.2 and 5.3 are expressed in the elliptical coordinates (ξ, η) , for which (ξ_0, η_0) corresponds to (x_0, y_0) ; $\xi = \xi_b$ is the boundary ellipse, $c e_{2n}(\eta, q)$ and $C e_{2n}(\xi, q)$ are the standard and modified Mathieu functions of the first kind, of order $2n$, and $\{q_{2n,r}\}$ are the zeros of $C e_{2n}(\xi_b, q)$. Definitions of these terms, and an outline of the method of solution, are given in Mahler (1985a).

In the case at hand, P_{O_2} was measured at or very near the midpoint of the lower border of a transverse section of the muscle, thus very near the center of the best-fitting hemi-ellipse (Fig. 2). For this case, $(x_0 = 0 = y_0)$, and for the boundary ellipse specified by the elliptical coordinate $\xi_b = 0.4$ (chosen for convenience, and for which $2m/l$, the width/thickness ratio, calculated by $l/m = \tanh \xi_b$, is 5.26), the values of $C_{n,r}$ and $k_{n,r}$ were evaluated for the first 10 terms of the double sum of Eq. 5.1 (Mahler, 1985a). The relative values of these $\{k_{n,r}\}$ are given in Table I. For each muscle, before the first contraction,

the term $k_{0,1}$ was measured as the rate constant of the final monoexponential phase of the time course of the P_{O_2} at the muscle surface measured by Method II of Mahler (1978a) (cf. Eq. 16 below). The other values of $k_{n,r}$ were then calculated by assuming that their values relative to $k_{0,1}$ were the same as for the case $\xi_b = 0.4$. The values of $C_{n,r}$ were assumed to be the same as for $\xi_b = 0.4$. The errors introduced by these assumptions were negligible; the best-fitting values of $2m/l$ determined on a separate set of muscles (cf. Results) had a mean of 5.06 ± 0.19 ($n = 18$), which corresponds to a value of 0.418 for ξ_b . These 10 terms, together with a convergence-speeding routine, used first with respect to n for a given r and then with respect to r , were used to evaluate $H(0, 0, j\omega)$ in the calculation of $\Delta Q_{O_2}(t)$ via Eq. 4 (Mahler, 1985a). All experiments were done at 20°C.

The oxygen electrode current at the end of recovery usually agreed closely, but not exactly, with its precontraction value. These slight discrepancies may have been due to electrode drift, altered basal Q_{O_2} (Mahler, 1978b), or a repositioning of the muscles. For the calculation of $\Delta Q_{O_2}(t)$, a linearly increasing correction was applied to the measured time course of electrode current, so that its final and initial values were identical. This had negligible effect on $\Delta Q_{O_2}(t)$, except after it had fallen to ~10% of its peak value, and made possible the calculation of $\int_0^\infty \Delta Q_{O_2}(t)dt$ by ensuring that $\Delta Q_{O_2}(t)$ returned to zero.

TABLE I
Relative Values of $k_{n,r}$ for $\xi_b = 0.4$

n	$r = 1$	$r = 2$	$r = 3$
0	1.00	7.494	20.11
1	2.288	10.22	24.35
2	4.327	13.58	29.18
3	7.174	—	—

Measurement of Solubility of O₂ in Muscle

These experiments were performed on resting muscles that had a constant rate of oxygen consumption. The principle of the method was to measure the extra oxygen uptake by the muscle (ΔO_2) caused by an increase in the P_{O_2} at its surface (ΔP). The solubility of oxygen in the muscle (α_m) could then be calculated as

$$\alpha_m = \frac{\Delta O_2}{\Delta P \cdot V_m}, \quad (6)$$

where V_m denotes the muscle volume.

A chamber essentially identical to that of Kushmerick and Paul (1976a) was constructed (see their Fig. 1). The oxygen electrode inserted into the chamber was of the type described by Mahler (1978a), with a 25- μ m platinum cathode. It was used with a polarographic current amplifier having an offset capability of 99.9 nA, in steps of 0.1 nA (model SV-400, Schema Versatae, Berkeley, CA), whose output was displayed on a chart recorder. In these experiments, it was necessary to measure a drop of ~3% in the chamber P_{O_2} . To reduce the electrode noise to an acceptable level, it was necessary to use three polyethylene membranes of 25.4 μ m thickness (0.001 in.), which slowed the electrode considerably. Its response to a step change in P_{O_2} at 22°C could be roughly approximated by a first-order model with $\tau = 62.8 \pm 5.0$ s ($n = 6$); a much better fit was obtained with a second-order model having $\tau_1 = 10.6 \pm 3.0$ s and $\tau_2 = 50.1 \pm 3.2$ s.² To minimize the

² A second-order system whose step response shows no oscillation reduces to a cascade of two first-order systems. If the latter have gains g_1 and g_2 and time constants τ_1 and τ_2 , the unit step response is $g_1 g_2 \{1 - [k_2/(k_2 - k_1)]e^{-k_1 t} + [k_1/(k_2 - k_1)]e^{-k_2 t}\}$, where $k_1 = 1/\tau_1$ and $k_2 = 1/\tau_2$. This was used to fit the electrode step response, with fitting parameters ($g_1 g_2$), k_1 , and k_2 .

effect of small variations in temperature on the electrode current, the chamber was immersed in a water bath whose temperature was set at the start of each day. The temperature at which the experiments were done ranged from 21.4 to 22.1°C. The linearity and stability of the O₂ electrode were checked before each day's experiments.

Sartorius muscles of *R. pipiens* were dissected, cut free of the pelvic bone, and allowed to recover in Ringer solution for 1–4 h at 4°C. Immediately before an experiment, a muscle was drained, mounted in preparation for insertion into the chamber, and then placed for 10–15 min in an auxiliary chamber, which was also immersed in the water bath and through which a rapid flow of air was maintained. From the results of previous experiments (Mahler, 1978a), it had been calculated that this was long enough to ensure that the intramuscular P_{O₂} profile had reached a steady state, provided Q_{O₂} was constant. The chamber, open at the top, was filled with Ringer solution, which was rapidly stirred and equilibrated with N₂, air, and 95% O₂/5% CO₂, in that order, added through small-bore polyethylene tubing. When the electrode trace had become stable in 95% O₂, the sensitivity of the amplifier was increased 10-fold, and the electrode current was offset sufficiently to allow full-scale recording. When the electrode trace again became stable, an experiment was begun. At time *t* = 0, the tubing through which gas was being added to the chamber was removed, and, virtually simultaneously, the top seal of the chamber, with the muscle mounted beneath, was inserted into the chamber. Special care was taken to ensure that no gas bubbles were trapped in the chamber.

To introduce the method of calculating α , consider first an idealized experiment of the type just described, done with a nonrespiring sheet of tissue. Let *P*₀ denote the P_{O₂} of the auxiliary chamber in which the tissue is initially gassed, and let *P*₁ be the P_{O₂} of the experimental chamber immediately before the insertion of the tissue. The P_{O₂} profile within the tissue would change as indicated in Fig. 1A. Initially, the P_{O₂} is constant at *P*₀. Placing the tissue in the Ringer-filled chamber raises the boundary P_{O₂} to *P*₁, and O₂ begins to enter the tissue. The lower dashed line shows an approximate P_{O₂} profile soon after *t* = 0, and the upper dashed line shows a later profile. Eventually, a new steady state will be reached, with the P_{O₂} everywhere in the chamber constant at *P*₁ - Δ, where Δ designates the overall drop in the P_{O₂} of the bathing medium caused by the flow of O₂ into the muscle. The measured time course of the P_{O₂} of the bathing medium would have the general form shown in Fig. 1B, allowing the measurement of Δ. The amount of O₂ taken up by the muscle during the experiment is

$$[(P_1 - \Delta) - P_0]\alpha_m V_m, \quad (7.1)$$

where α_m and *V*_m denote the solubility of O₂ in the muscle and the muscle volume, respectively. On the other hand, the amount of O₂ leaving the bath is

$$\Delta\alpha_R V_R, \quad (7.2)$$

where α_R and *V*_R denote the solubility of O₂ in Ringer solution and the volume of Ringer solution, respectively. Equating Eqs. 7.1 and 7.2 and solving for α_m yields

$$\alpha_m = \frac{\Delta\alpha_R V_R}{(P_1 - P_0 - \Delta)V_m}; \quad (7.3)$$

and solving for Δ yields

$$\Delta = (P_1 - P_0)/[1 + (\alpha_R V_R/\alpha_m V_m)]. \quad (7.4)$$

The extension of these principles to the case of a tissue consuming oxygen at a constant rate can best be explained using a typical experimental record (Fig. 1C). Once the muscle enters the chamber, its O₂ uptake, indicated by the declining P_{O₂} of the external medium,

is determined, via the diffusion equation, by two factors: the initial change in the P_{O_2} at the muscle surface, and the muscle Q_{O_2} . Intuitively, one can expect the effect of the initial change in surface P_{O_2} to be transient, with a time course roughly similar to that in the absence of tissue respiration (Fig. 1B), and once this process is complete, the constant

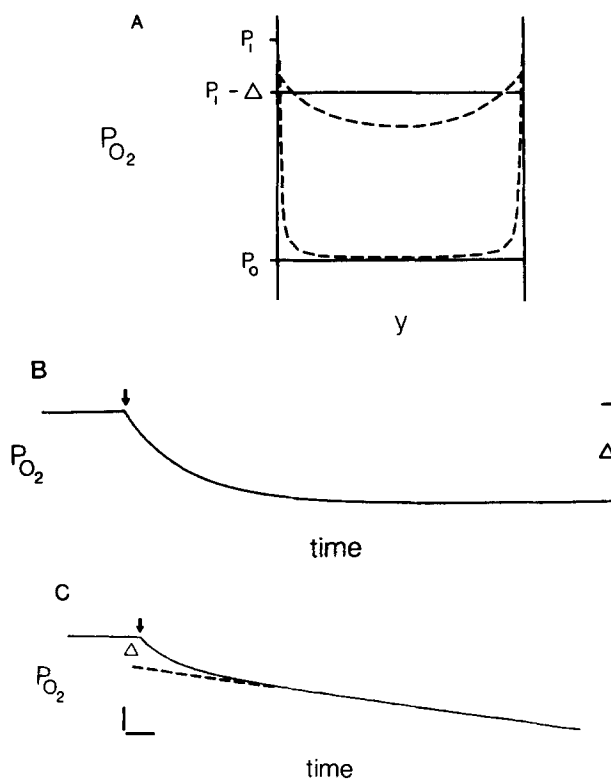


FIGURE 1. (A) P_{O_2} within a nonrespiring sheet contained in a closed chamber, as a function of distance (y) from the surface, for an idealized experiment in which the boundary P_{O_2} is changed from P_0 to P_1 at time $t = 0$. The solid lines indicate the initial and final profiles, and the dashed lines indicate intermediate profiles. See text for further details. (B) Time course of chamber P_{O_2} during the idealized experiment described in A, showing the graphical determination of Δ , the overall drop in chamber P_{O_2} . (C) A typical experimental record of the time course of chamber P_{O_2} during a determination of the solubility of oxygen in a frog sartorius muscle. Scale bars indicate 14.2 mmHg and 2 min. Extrapolation of the final linear phase of the record back to $t = 0$ allows the graphic determination of the quantity Δ described in Eq. 7.4.

tissue Q_{O_2} , would be expected to cause a linear decrease in the P_{O_2} of the external medium. Fig. 1C shows that the experimental records were consistent with this expectation. Moreover, as shown in the Appendix, if the linear portion of the record is extrapolated back to $t = 0$, its intercept, denoted Δ , has the same value it would have in the absence of tissue respiration (Fig. 1B, Eq. 7.4), enabling α_m to be calculated via Eq. 7.3.

The values used for α_R , the solubility of O_2 in frog Ringer solution, at the various

experimental temperatures were obtained by linear interpolation from the values of α in 0.119 N NaCl at 21–23°C given by Bartels et al. (1971). V_R , the volume of Ringer solution in the chamber, was determined by an O₂ dilution technique. The chamber was filled with water that had been equilibrated with room air, and sealed. When the O₂ electrode trace became stable, 100 μ l of water that had been equilibrated with 100% N₂ was injected into the chamber with a Hamilton syringe, thereby displacing an equal volume of the solution initially present, and the drop in P_{O_2} was measured. This procedure also furnished the response of the O₂ electrode to a step change in P_{O_2} . For heuristic purposes, this response can be represented by Fig. 1B, and, using its terminology, the chamber volume, in milliliters, is $0.1 P_1/\Delta$; this was 4.21 ± 0.07 ml ($n = 16$). To obtain the value of V_R used in Eq. 7.3, it was necessary to subtract the volume of the muscle in its drained state, as it was on insertion into the chamber. This was assumed to be $M_{bl}[(1/\rho) + 0.089 \text{ cm}^3/\text{g}]$, where M_{bl} denotes the blotted weight of the muscle and ρ is its density (1.06 g/cm³, Gore and Whalen, 1968); the quantity $0.089 M_{bl}$ represents the weight of Ringer solution adhering to the muscle in the drained state, based on a large number of previous measurements.

The value of Δ , determined graphically as in Fig. 1C, was corrected for the error introduced by the O₂ electrode. As noted above, the response characteristics of the electrode were well described as a second-order system. If the output of such a system has the form shown in Fig. 1C, its input, the actual time course of P_{O_2} at the electrode, must also have a final linear phase with the same slope, leading that of the output by $\tau_1 + \tau_2$, which in this case was 60.7 ± 4.7 s ($n = 6$). The observed Δ thus underestimates the true value by the amount the linear phase of the record falls in 60.7 s; this correction averaged $5.4 \pm 0.3\%$ ($n = 9$) of the observed value. The corrected value of Δ was denoted Δ_{corr} . The full formula used to calculate the solubility of oxygen in the drained muscle was thus

$$\alpha_{m,\text{drained}} = \frac{\Delta_{\text{corr}} \alpha_R V_R}{(P_1 - P_0 - \Delta_{\text{corr}}) M_{bl} [(1/\rho) + 0.089 \text{ cm}^3/\text{g}]} \quad (7.5)$$

To calculate the solubility of oxygen in the blotted muscle itself, as distinct from the Ringer solution adhering to it in the drained state on insertion into the chamber, it was necessary to correct for the oxygen taken up by this adhering Ringer solution. The formula used was

$$\alpha_m = \frac{\Delta_{\text{corr}} \alpha_R V_R - (P_1 - P_0 - \Delta_{\text{corr}}) \alpha_R M_{bl} (0.089 \text{ cm}^3/\text{g})}{(P_1 - P_0 - \Delta_{\text{corr}}) (M_{bl}/\rho)} \quad (7.6)$$

In a few preliminary experiments, muscles were initially equilibrated with 95% O₂ and then transferred to the chamber, which was filled with Ringer solution equilibrated with air. Once the muscle had entered the chamber, the decrease in its O₂ store caused by the decrease in external P_{O_2} could be determined graphically by a procedure analogous to that described above (cf. Fig. 6). However, because the muscle also lost a small amount of O₂ during its transfer to the chamber, this methodology was abandoned in favor of the one described above.

Comparison of Direct and Diffusion Methods

MEASUREMENT OF Q_{O_2} BY THE DIRECT METHOD These experiments were done with a chamber of the type used by Kushmerick and Paul (1976a), described above. The characteristics of the oxygen electrode, preparation of the muscle, and expansion of the P_{O_2} scale were also as described above. All experiments were done at 20°C.

RESTING $Q_{O_2}(Q_0)$ The Ringer solution in the chamber was initially equilibrated with air. The chamber was then sealed, to check the basal drift of the chamber P_{O_2} -recording

system. This was described by the best-fitting line, with slope denoted s_e , which averaged $\sim 0.05\%/min$, or if expressed as a loss of O₂, 0.08 mmHg/min. After a muscle was placed in the chamber, the chamber P_{O_2} trace became linear within ~ 20 min, with slope denoted s_o , which averaged ~ 0.5 mmHg/min. Once the trace had been linear for 10 min, the muscle was removed from the chamber, blotted, weighed, and then frozen and stored under liquid N₂. The total creatine (C_T) content of the muscles was measured by the method of Homsher et al. (1972).

Q_0 was calculated as

$$Q_0 = (S_0 - S_e)(\alpha_R V_R + \alpha_m V_m)/M_{bl}. \quad (8)$$

Q_0 is the sum of the rates of decrease in the O₂ contents of the chamber and of the muscle itself. When the P_{O_2} trace is linear, the former quantity is $(s_0 - s_e)\alpha_R V_R$. From the exact solution of Eq. 1 for a plane sheet contained in a sealed chamber (Louy, 1983; cf. Appendix), it follows that the rate of decrease in the O₂ content of the tissue asymptotically approaches $(s_0 - s_e)\alpha_m V_m$. By analogy, this relationship should also be valid for any other symmetrical tissue configuration. Under the present experimental conditions, $\alpha_m V_m$ was only $\sim 2\%$ as large as $\alpha_R V_R$. This exact solution also predicts that during the linear phase of the P_{O_2} trace, the P_{O_2} drop from the medium to the center of the muscle is well approximated by the value that would exist in a true steady state with the same external P_{O_2} . For the analogous case of an elliptical tissue, this is

$$\frac{Q_0}{2D\alpha} \cdot \frac{l^2 m^2}{l^2 + m^2} \quad (9)$$

(A. V. Hill, 1966). This averaged ~ 15 mmHg, which indicated that the muscles were adequately oxygenated throughout the experiments.

STEADY STATE INCREASE IN Q_{O_2} (ΔQ_{ss}) The Ringer solution in the chamber was initially equilibrated with 60.5% O₂. A resting muscle was inserted into the chamber. Once the P_{O_2} trace had become linear, and had remained so for 10 min, the muscle was given a single supramaximal stimulus of 0.6 ms duration every 12 s. The rate of decrease in chamber P_{O_2} began to change noticeably after ~ 3 min of stimulation (Fig. 7A) and became linear after ~ 15 min, with slope denoted s_1 . Once the trace had remained linear for 10 min, stimulation was ceased. After ~ 15 min, the P_{O_2} trace returned to a basal, linear state. The muscle was then removed, blotted, weighed, and then frozen to await determination of {C_T}. The average of the pre- and poststimulation basal slopes was denoted s_0 . By exact analogy with the calculation of Q_0 (Eq. 8), ΔQ_{ss} was calculated by

$$\Delta Q_{ss} = (s_1 - s_0)(\alpha_R V_R + \alpha_m V_m)/M_{bl}. \quad (10)$$

TOTAL O₂ CONSUMPTION AFTER A TETANUS ($\Delta\{O_2\}$) The initial methodology was as described in the preceding section. The muscle was tetanized by a train of just-supramaximal stimuli of 0.6 ms duration and 70/s frequency for either 0.2 or 0.5 s. After stimulation, the chamber P_{O_2} followed a curvilinear path (Fig. 7B), becoming linear again after ~ 15 min. As described by Kushmerick and Paul (1976a), two estimates were made of the total drop in P_{O_2} caused by the tetanus (denoted ΔP_t), one by extrapolating the final linear phase back to time of stimulation ($t = 0$), the other by extrapolating the initial linear phase ahead 16–22 min. If these estimates did not agree to within 20%, the record was discarded; if the records further analyzed, the two estimates usually agreed to within 5%. $\Delta\{O_2\}$ was calculated as

$$\Delta\{O_2\} = \Delta P_t(\alpha_R V_R + \alpha_m V_m)/M_{bl}. \quad (11)$$

TIME COURSE OF Q_{O_2} AFTER A TETANUS With the method of Louy (1983), the time course of Q_{O_2} in a sheetlike tissue can be calculated from the time course of P_{O_2} in a closed

chamber containing the tissue, as measured by the direct method. On the basis of the results of Mahler (1978*b*), $\Delta Q_{O_2}(t)$ after an isometric tetanus was assumed to have the form $\Delta Q_0 + \Delta Q_1 (e^{-t/\tau_1} - e^{-t/\tau_2})$. This included the case $Qe^{-t/\tau}$ postulated by Kushmerick and Paul (1976*a*). The precontraction basal Q_{O_2} was denoted Q_0 . The diffusion equation (Eq. 1) was then solved exactly to yield the predicted form of the time course of chamber P_{O_2} (for details, see Louy, 1983). A nonlinear least-squares regression program was used to determine the values of Q_0 , ΔQ_0 , ΔQ_1 , τ_1 , and τ_2 that provided the best fit to the time course of chamber P_{O_2} actually observed. The response characteristics of the oxygen electrode (see above) were taken into account in this calculation. For the frog sartorius, the tissue was assumed to be sheetlike, whereas the freely suspended muscle was probably well described as an elliptical cylinder with $m/l \approx 5$. However, by analogy with the diffusion method results (see Fig. 3*B*), the use of the sheet model presumably furnishes a useful approximation to the result obtained with this elliptical boundary.

MEASURE OF Q_{O_2} BY THE DIFFUSION METHOD These experiments were done with the chamber described in Mahler (1978*a, b*). The oxygen electrode was covered with a single polyethylene membrane of 25.4 μm thickness or with two membranes of 12.7 μm thickness. The response of the electrode to a step change in P_{O_2} was adequately described by a single exponential with time constant of ~ 8 s. All experiments were done at 20°C.

Q_0 The chamber gas contained either 60.5% or 95% O_2 . The steady state drop in P_{O_2} (ΔP_0) from the gas phase to the center of the closed, lower surface of the muscle was measured by Method I of Mahler (1978*a*), in which the lower surface is closed by raising the electrode against it. With a 25.4- μm membrane, when the electrode was placed in contact with the muscle, the small, sudden drop in electrode current observed by Mahler (1978*a*) when using a single 12.7- μm membrane did not occur. These experiments also provided the rate constant (k) of the final, monoexponential phase of the time course of P_{O_2} at the closed surface before the attainment of a steady state.

Using $m/l = 2.53$ (see Results), the steady state solution of the diffusion equation for an elliptical cylinder (Eq. 9) predicts that

$$Q_0 = 2.312\Delta P_0 D\alpha/l^2. \quad (12)$$

Substituting Eq. 17 into Eq. 12 yields

$$Q_0 = 0.708\Delta P_0 k\alpha, \quad (13)$$

which was used to calculate Q_0 . (Contrast Eqs. 12 and 13 with Eqs. 8 and 9 of Mahler [1978*a*], which were based on the sheet model.) The value of α used in evaluating Eq. 13 (and Eqs. 5.2 and 14) was 0.03846 ml $O_2/\text{cm}^3 \cdot \text{atm}$, based on the result $\alpha(\text{muscle})/\alpha(\text{H}_2\text{O}) = 1.24$ for drained muscle (see Results), and a value of 0.03102 ml $O_2/\text{cm}^3 \cdot \text{atm}$ for $\alpha(\text{H}_2\text{O})$ at 20°C (Bartels et al., 1971).

ΔQ_{ss} After a steady state had been attained in a Method I experiment using 95% O_2 , the muscle was given a single supramaximal stimulus of 0.6 ms duration every 12 s. The P_{O_2} at the closed surface followed an S-shaped time course, reaching a new constant value after 30 min (Fig. 7*C*). The muscle was then removed from the chamber, blotted, weighed, frozen, and stored for determination of C_T content.

The drop from its resting value in the steady state P_{O_2} at the center of the closed surface was denoted ΔP_1 , and by exact analogy with the calculation of Q_0 , ΔQ_{ss} was calculated by

$$\Delta Q_{ss} = 0.708\Delta P_1 k\alpha. \quad (14)$$

$\Delta\{O_2\}$ $\Delta\{O_2\}$ was calculated as $\int_0^\infty \Delta Q_{O_2}(t)dt$. After a steady state had been attained in a Method I experiment using 60.5% O_2 , the muscle was tetanized by a train of just-

supramaximal stimuli of 0.6 ms duration and 70/s frequency for 0.2 s. $\Delta Q_{O_2}(t)$ was measured as described above.

Other Methods

Rapid freezing of stimulated muscles was done by the method of Homsher et al. (1975). The preparation of muscle extracts and the measurement of the contents of creatine, PC, ATP, ADP, AMP, and lactate in the extracts was done by the methods of Homsher et al. (1972). The measurement of muscle heat production during and after an isometric tetanus was done by the method of Homsher et al. (1975).

RESULTS

Quantification of Tissue Configuration

The central portion of a transverse section of the frog sartorius was usually well fit by a hemi-ellipse (Fig. 2). The mean value of $2m/l$, i.e., the width divided by the maximum thickness, for the best-fitting ellipses was 5.06 ± 0.19 ($n = 18$).

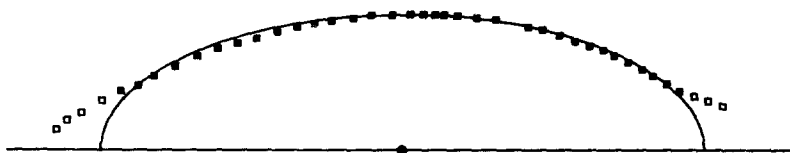


FIGURE 2. Typical fit by a hemi-ellipse of points digitized from the upper surface of a transverse section of a frog sartorius muscle. The points designated by unfilled symbols were neglected in this fit (see text for details of fitting procedure).

Calculation of Diffusion Coefficient

It was previously shown (Mahler, 1978a) that after a step-like change in the P_{O_2} at the upper surface of the excised frog sartorius, the change in P_{O_2} at the center of its closed, lower surface follows an S-shaped time course that relatively quickly becomes monoexponential. This entire time course could be well fit by the appropriate solution of the one-dimensional diffusion equation, which is

$$\Delta P(0, t) = (P_1 - P_0) \left\{ 1 - \frac{4}{\pi} \sum_{n=0}^{\infty} \frac{(-1)^n}{(2n+1)} e^{-[(2n+1)^2 \pi^2 D/4l^2]t} \right\}, \quad (15)$$

where P_0 and P_1 are the initial and final values of P_{O_2} at the upper surface. However, the corresponding solution of the two-dimensional diffusion equation for a hemi-elliptical boundary proved to have the same general form as the solution for a sheet. It is

$$\Delta P(0, 0, t) = (P_1 - P_0) \left[1 + \sum_{n=0}^{\infty} \sum_{r=1}^{\infty} C_{n,r} e^{-k_{n,r}t} \right], \quad (16)$$

where $C_{n,r}$ and $k_{n,r}$ are given in Eqs. 5.2 and 5.3 (Mahler, 1985a). This expression also provided an excellent fit of the measured time course of P_{O_2} , which thus remains consistent with the hypothesis that the diffusion equation is valid on the

macroscale in this tissue. However, the best-fitting value of the diffusion coefficient D is considerably different for the hemi-elliptical model than for the sheet model. If k denotes the rate constant of the monoexponential phase of the recorded time course of P_{O_2} , rearranging Eq. 5.3 (in which $k_{0,1} = k$) yields

$$D = kl^2/q_{0,1}4\sinh^2\xi_b. \quad (17)$$

On the other hand, it follows from Eq. 15 that the sheet model predicts that $D = kl^2/(\pi^2/4)$. For the case at hand, using $2m/l = 5.06$ and $\xi_b = \tanh^{-1}(l/m)$, ξ_b is 0.418. With the methods of Mahler (1985a), the corresponding value of $q_{0,1}$ was found to be 4.408, for which $q_{0,1}4\sinh^2\xi_b = 3.264$; thus, for this configuration, the value obtained for D via Eq. 17 is 32.3% smaller than that obtained with the sheet model. Table II gives revised estimates of D , based on this average value of ξ_b , for 22.8, 10, and 0°C, calculated from the results of Mahler

TABLE II
Values of the Diffusion Coefficient (D), Solubility (α), and the Krogh Permeation Constant ($K = D\alpha$) for Oxygen in Frog Sartorius Muscle

T	$D \times 10^5$	α	$K \times 10^5$
°C	cm ² /s	ml O ₂ /cm ³ ·atm	ml O ₂ /cm·min·atm
0	0.622±0.020 (17)	0.0606	2.26
10	0.794±0.038 (10)	0.0471	2.24
20	1.01	0.0384	2.33
22.8	1.08±0.043 (12)	0.0365±0.0011 (9)	2.37±0.12 (13.6)
37	1.45	0.0296	2.57

Measured values are given ± SEM (degrees of freedom). Other values of D were obtained by interpolation or extrapolation. Other values of α were obtained by assuming that $\alpha/\alpha(\text{H}_2\text{O})$ was the same for all values of T .

(1978a). Linear interpolation gave a value of 1.01×10^{-5} cm²/s at 20°C. In a few experiments, k , l , and ξ_b (range, 0.349–0.452) were measured on individual muscles at 20°C, yielding a value for D of 0.94 ± 0.07 ($n = 4$) $\times 10^{-5}$ cm²/s. Using the range of published values for the diffusion coefficient of O₂ in water (cf. Mahler, 1978a), $D(\text{muscle})/D(\text{H}_2\text{O})$ was in the range 0.45–0.54. The calculated average activation energy for diffusion of O₂ in muscle (–3.85 kcal/mol, Mahler, 1978a) was not affected by the revisions in the values of D .

Time Course of Q_{O_2} After a Tetanus

Fig. 3B shows, for a typical experiment employing a brief isometric tetanus, the difference between the time course of ΔQ_{O_2} calculated using the hemi-elliptical cylinder model and that calculated using the sheet model. Several general features are of interest. First, the elliptical cylinder model yields lower values than the sheet model for ΔQ_{O_2} . At first glance, this seems paradoxical, since an analysis of the effects of tissue configuration per se predicts the opposite result (cf. Discussion). Second, the general form of the time course of ΔQ_{O_2} was essentially the same with both models. Mahler (1978b, 1979) reported that after a tetanus of 0.1–1.0 s in the sartorius of *R. pipiens* at 20°C, ΔQ_{O_2} rose rapidly to a peak, reached within 45–90 s, and that its subsequent decline was usually well

fit by a single exponential, with τ having a median value of ~ 3 min. With the elliptical cylinder model, the rise of ΔQ_{O_2} was essentially unchanged, and its descending limb was again well fit by a monoexponential, with $\tau \sim 15\%$ smaller than the value calculated with the sheet model. The fit shown in Fig. 4A was typical for a 0.5-s tetanus of the sartorius of *R. temporaria* at 20°C.

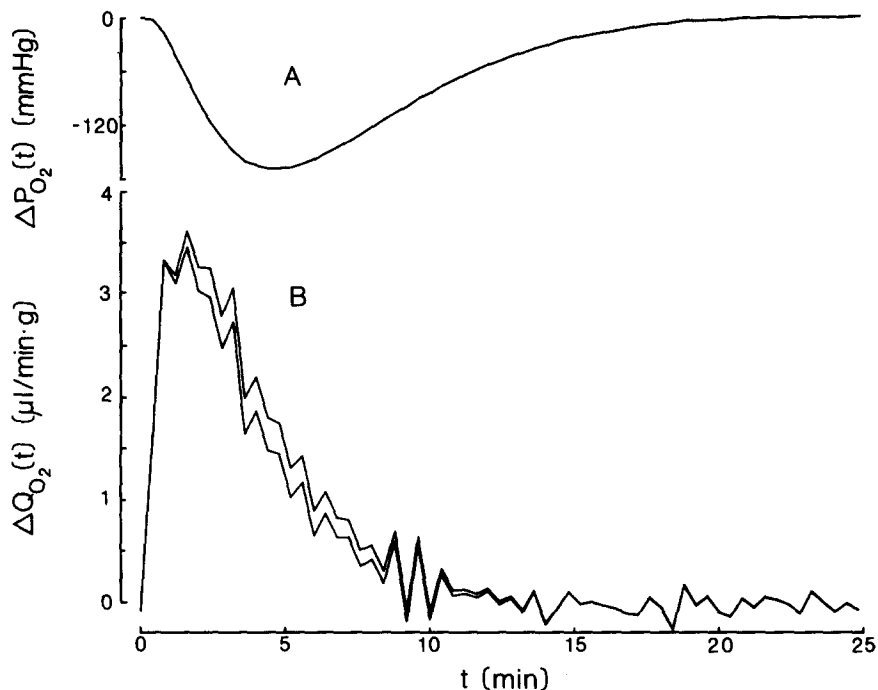


FIGURE 3. (A) Typical time course of change in the P_{O_2} at the closed, lower surface of a sartorius of *R. temporaria* after a tetanus of 0.5 s at 20°C. Points were digitized from the experimental record at 24-s intervals and are connected here by line segments. (B) Upper curve: time course of ΔQ_{O_2} calculated from the points shown in A by the methods of Mahler (1978a, b), in which the tissue is treated as a plane sheet. The discrete values of ΔQ_{O_2} have been connected by line segments. Lower curve: time course of ΔQ_{O_2} calculated by the method described in this paper, in which the tissue is treated as a hemi-ellipse.

To further investigate the suitability of a monoexponential fit of the descending limb of $\Delta Q_{O_2}(t)$, the oscillations inherent in the individual records (Mahler, 1978b) were minimized by averaging all the records of given series. In each case, the mean response was still well described by a single exponential. For a 0.5-s tetanus in the sartorius of *R. temporaria*, the best-fitting value of τ was 2.12 min (Fig. 4B). In the sartorius of *R. pipens*, τ was significantly larger: for a recent series of 0.2-s tetani, τ was 2.75 min, and when the data of Mahler (1978b) for 0.5-s and 1.0-s tetani were re-analyzed with the elliptical cylinder model and averaged, the respective values of τ were 2.76 and 2.46 min. As previously concluded using the sheet model (Mahler, 1978b), for individual muscles no

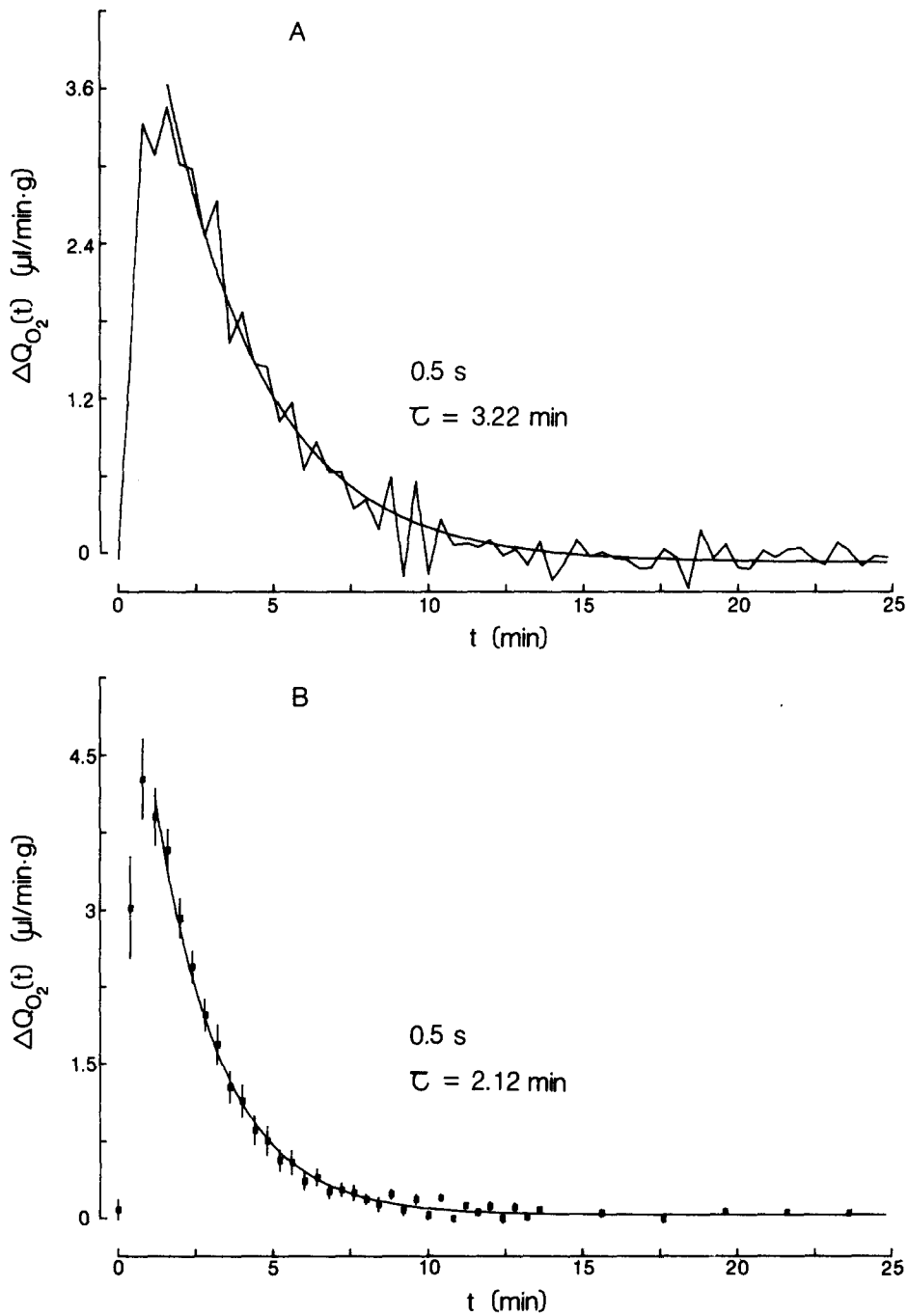


FIGURE 4. (A) Time course of ΔQ_{O_2} as shown in the lower curve of Fig. 3B, together with the curve of the form $a + be^{-t/\tau}$ that best fits the descending limb of $\Delta Q_{O_2}(t)$, and for which $\tau = 3.22$ min. (B) Mean of 13 records of the type shown in A, for a tetanus of 0.5 s in the sartorius of *R. temporaria* at 20°C. The smooth curve is the best-fitting monoexponential, for which $\tau = 2.12$ min.

dependence of τ on tetanus duration could be demonstrated for tetani of 0.1–1.0 s.

To test the accuracy of the form of the time course of Q_{O_2} calculated with the diffusion method and the elliptical cylinder model, we compared it with the time course of the rate of heat production under the same conditions. D. K. Hill (1940*a*), using a differential volumeter, reported the form of the time course of oxygen consumption in the sartorius of *R. temporaria* after a single tetanus at 0°C, and subsequently showed that the time course of recovery heat production had virtually the same form (D. K. Hill, 1940*b*). Fig. 5*A* shows the mean time course of suprabasal recovery heat production, denoted $\Delta h(t)$, in the sartorius of *R. temporaria* after a 0.5-s tetanus at 20°C, together with the corresponding rates of heat production, $\Delta \dot{h}(t)$. In Fig. 5*B*, the latter values are plotted on a nondimensionalized scale, together with the mean values of $\Delta Q_{O_2}(t)$ from companion experiments on different muscles. The close agreement between the two suggests that the form of $\Delta Q_{O_2}(t)$ determined with the diffusion method is highly accurate.

Solubility of O₂ in Muscle

Because the values of α yielded by these experiments were so much greater than the estimate made by A. V. Hill (1966), a sample calculation based on the record shown in Fig. 1*C* is given below. At $t = 0$, the P_{O_2} of the external medium was changed from 154.0 to 699.1 mmHg. This caused an apparent drop (Δ) of 19.1 mmHg in the P_{O_2} of the chamber, whose volume was 4.21 cm³. After correcting for the response characteristics of the oxygen electrode, the actual drop in chamber P_{O_2} was calculated to be 20.2 mmHg. The blotted muscle weighed 142.5 mg, so its volume was 0.134 cm³, and that of the 95% O₂-equilibrated Ringer solution in the chamber at $t = 0$ was 4.063 cm³. The temperature of the chamber was 21.4°C, at which α_R is 0.02935 ml O₂/cm³·atm. For these figures, Eqs. 7.5 and 7.6 yielded values for α_m of 0.0312 and 0.0314 ml O₂/cm³·atm, respectively, for the drained and blotted states. At this temperature, α_{H_2O} is 0.03022 ml O₂/cm³·atm (Bartels et al., 1971). For 13 experiments on 10 muscles, α_m/α_{H_2O} had a mean of 1.24 ± 0.04 (SEM; $n = 9$) for the drained muscles, and 1.26 ± 0.04 for the blotted muscles.

An internal check of the methodology was provided by a few experiments in which the P_{O_2} of the external medium was decreased at $t = 0$ rather than increased. This protocol was perhaps the more graphic, since it demonstrated a large release of O₂ from the muscle (Fig. 6), but it was also less accurate (cf. Materials and Methods). These experiments yielded values for α_m/α_{H_2O} of ~ 1.1 .

Comparison of Direct and Diffusion Methods

Some typical results of the comparison of the two methods are shown in Fig. 7. Table III shows that when the diffusion method was used with the elliptical-cylinder model and the value of α reported here, it gave virtually the same values as the direct method for all three test quantities: the resting Q_{O_2} (Q_0), the steady state increase in Q_{O_2} (ΔQ_{ss}) for eight twitches per minute, and the total suprabasal O₂ consumption ($\Delta\{O_2\}$) after a 0.2-s tetanus. When the results were expressed

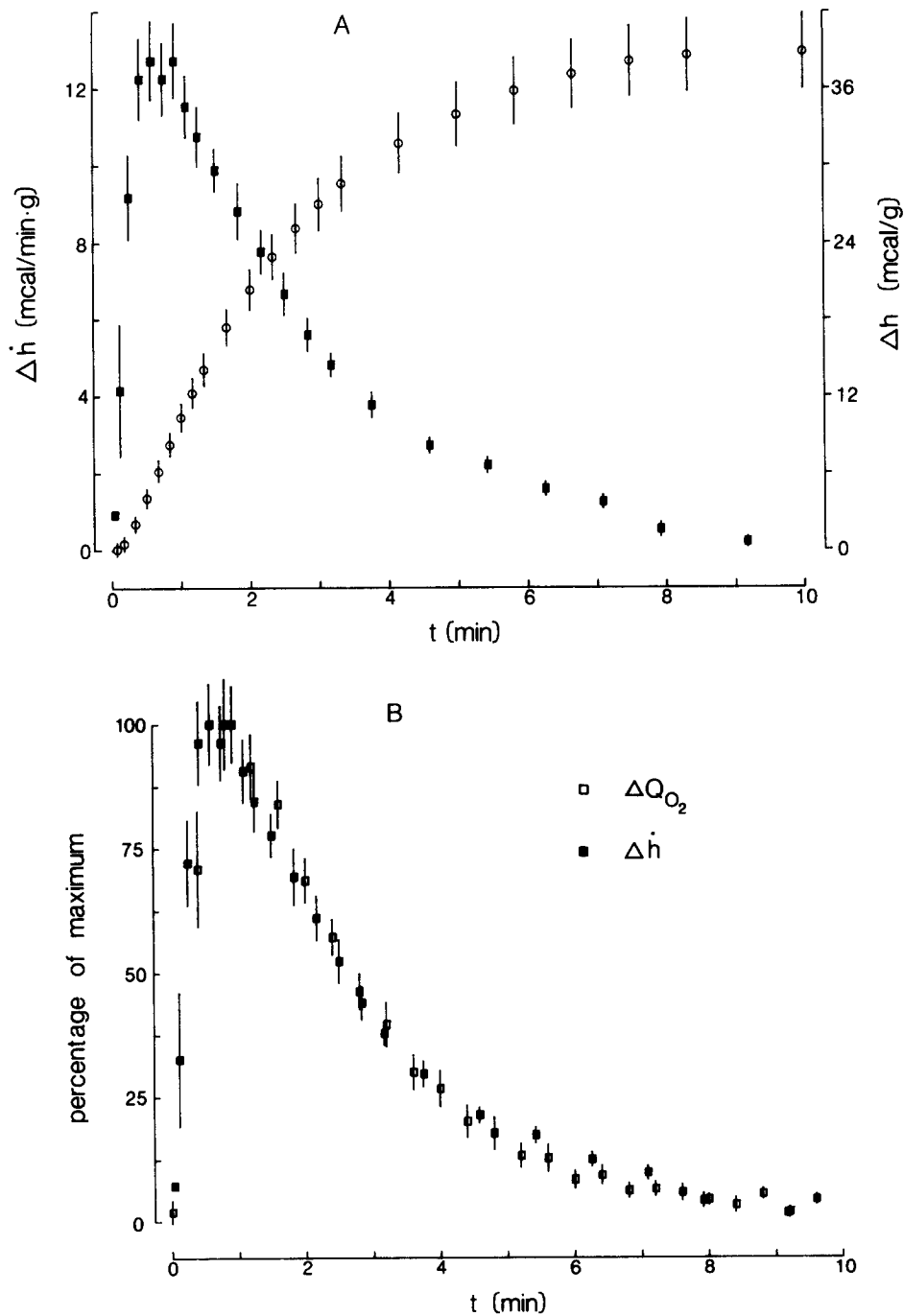


FIGURE 5. (A) Circles: mean time course of suprabasal recovery heat production (Δh) in the sartorius of *R. temporaria* after a 0.5-s tetanus at 20°C. Squares: simultaneous mean time course of the suprabasal rate of heat production, $\Delta \dot{h}$. This was calculated as the average rate for each time interval between measurements of Δh . For both curves, error bars indicate \pm SEM ($n = 8$). (B) Filled squares: nondimensionalized plot of the values of $\Delta \dot{h}(t)$ shown in A. Open squares: nondimensionalized plot of the mean time course of ΔQ_{O_2} as shown in Fig. 4B, measured by the diffusion method in a separate group of muscles under identical conditions.



FIGURE 6. Time course of P_{O_2} in a chamber initially equilibrated with air, after a muscle equilibrated with 95% O_2 was placed in the chamber at time $t = 0$, indicated by the arrow. Scale bars indicate 2.75 mmHg and 2 min. Extrapolation of the final linear phase of the record back to $t = 0$ gives an intercept Δ analogous to that of Fig. 1C.

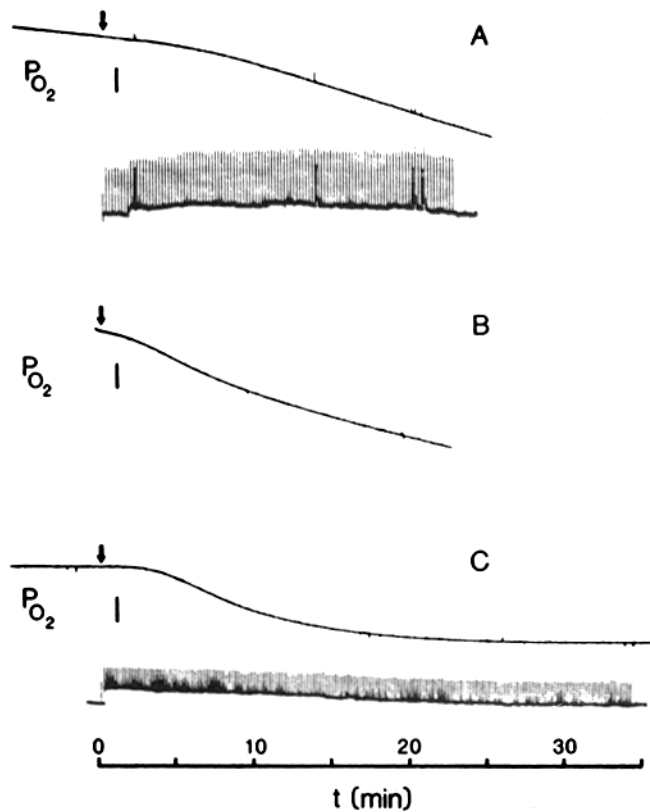


FIGURE 7. (A) Photograph of a typical time course of chamber P_{O_2} during measurement of ΔQ_{O_2} by the direct method. Beginning at the time indicated by the arrow, the muscle was stimulated once every 12 s. Scale bar indicates 9.30 mmHg. The record of twitch tension is displayed beneath that of P_{O_2} . (B) Typical time course of chamber P_{O_2} during measurement of $\Delta\{O_2\}$ by the direct method. At the time indicated by the arrow, the muscle was stimulated for 0.2 s. The scale bar indicates 4.44 mmHg. The precontraction slope (not shown) closely matched that at the end of recovery. (C) Typical time course of P_{O_2} at the closed, lower surface of a muscle during measurement of ΔQ_{O_2} by the diffusion method. Stimulation protocol and tension record are the same as in A. The scale bar indicates 141.0 mmHg.

in terms of muscle weight, the diffusion-method values were ~5–10% higher than the direct-method values, but after normalization by total creatine content ($\{C_T\}$; Carlson et al., 1967), these discrepancies disappeared. For a given comparison, all muscles were taken from the same batch of frogs, so the slightly higher values of $\{C_T\}$ after the diffusion-method experiments may have resulted from a loss of muscle water during prolonged exposure to a gaseous environment.

As shown in Fig. 8, the time course of ΔQ_{O_2} after a tetanus could also be estimated with the direct method. The measured time course of the chamber P_{O_2} was typically well fit by the solution of the one-dimensional diffusion equation when $\Delta Q_{O_2}(t)$ was assumed to have the form $\Delta Q_0 + \Delta Q_1 (e^{-t/\tau_1} - e^{-t/\tau_2})$ (Louy, 1983; Fig. 8A). For the best-fitting values of ΔQ_0 , ΔQ_1 , τ_1 , and τ_2 , $\Delta Q_{O_2}(t)$ (Fig. 8B) proved to be similar to that measured by the diffusion method and distinct

TABLE III
Comparison of Results of Direct and Diffusion Methods

	Direct method	Diffusion method
Q_0 (nmol/min·g)	32.0±1.7 (11)	35.8±2.0 (30)
$Q_0/\{C_T\}$ (nmol/min·μmol)	0.822±0.044 (11)	0.865±0.069 (20)
ΔQ_{μ} (nmol/min·g)	136±12 (10)	153±14 (8)
$\Delta Q_{\mu}/\{C_T\}$ (nmol/min·μmol)	3.59±0.31 (10)	3.55±0.29 (7)
$\Delta\{O_2\}$ (nmol/g)	320±17 (10)	335±22 (12)
$\Delta\{O_2\}/\{C_T\}$ (nmol/μmol)	7.75±0.29 (10)	7.42±0.47 (12)

g denotes gram blotted weight; C_T denotes total creatine content. Values are given ± SEM (degrees of freedom).

from the monoexponential form postulated by Kushmerick and Paul (1976a), which required that τ_2 be approximately equal to 0. It was of interest to compare $\Delta Q_{O_2}(t)$ with the time course of oxygen uptake by the muscle, which is the rate of change of chamber P_{O_2} (Fig. 8, B and C); the difference between the left-hand curves of Fig. 8, B and C, is the rate of change in the O_2 store of the muscle. In the present case, it was important to take into account the time response of the oxygen electrode.

$\Delta\{PC\}_0/\Delta\{O_2\}$ and Postcontractile Suprabasal ATP Hydrolysis

In view of the good agreement between the results obtained with the direct and diffusion methods for measuring Q_{O_2} , a large number of past and present results were combined to obtain estimates of the ratio $\Delta\{PC\}_0/\Delta\{O_2\}$ for a brief tetanus of the frog sartorius at 20°C and of the amount of suprabasal ATP hydrolysis during recovery (Table IV).

Energy balance studies showed that in well-oxygenated sartorii of *R. pipiens* or *R. temporaria*, a significant fraction of the energy liberation during a tetanus was not accounted for by the observed breakdown of PC, assuming a molar enthalpy for PC hydrolysis of 34 kJ/mol (Curtin and Woledge, 1978) (Table IV). No statistically significant changes were seen in the levels of ATP, ADP, AMP, or lactate. These results are quantitatively similar to those of a large number of

studies on frog skeletal muscle at 0°C (for review, see Curtin and Woledge, 1978; Homsher and Kean, 1978), and to those of Canfield et al. (1973) for *R. temporaria* at 20°C.

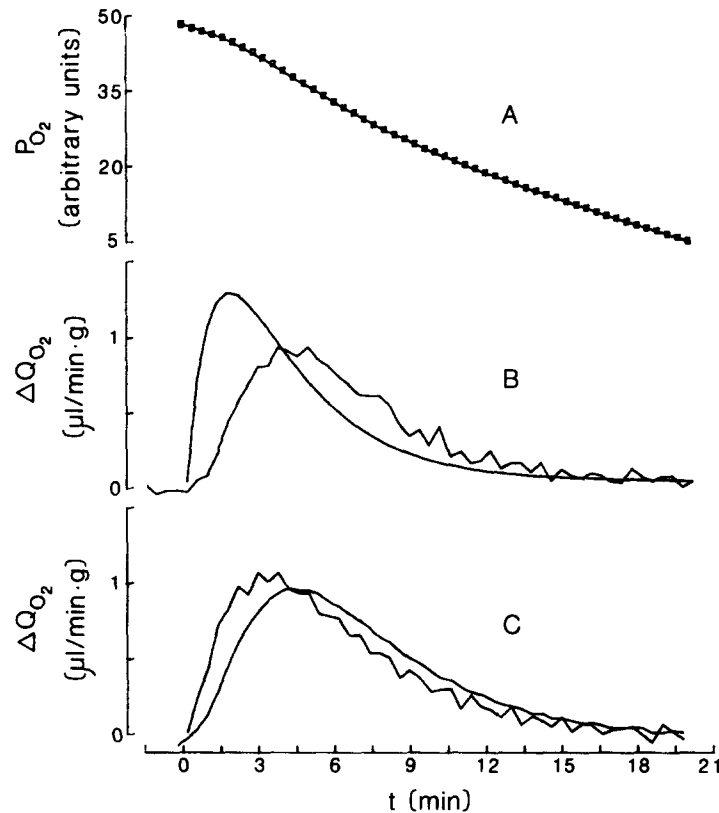


FIGURE 8. (A) Squares: time course of chamber P_{O_2} measured with the direct method after a tetanus of 0.2 s in the sartorius of *R. pipiens*. Smooth curve: best-fitting solution of the one-dimensional diffusion equation with $\Delta Q_{O_2}(t)$ having the general form $\Delta Q_0 + \Delta Q_1 (e^{-t/\tau_1} - e^{-t/\tau_2})$. ΔQ_0 , ΔQ_1 , τ_1 , and τ_2 are determined by the fitting procedure. (B) Left-hand curve: the $\Delta Q_{O_2}(t)$ that produced the fit shown in A. Right-hand curve: time course of the suprabasal rate of O_2 uptake from the chamber, calculated from the data in A as the average rates for the intervals between measurements of chamber P_{O_2} . (C) Right-hand curve: mean time course of the rate of O_2 uptake for 11 experiments of the type described in A and B. More precisely, this represents apparent O_2 uptake, since the delay imposed by the oxygen electrode has not been taken into account. When this is done, the left-hand curve results.

The reactions causing this unexplained enthalpy might be reversed after a contraction, at the expense of suprabasal ATP hydrolysis (Curtin and Woledge, 1978; Homsher and Kean, 1978). The present results provided two indirect methods of quantifying the total suprabasal ATP hydrolysis during recovery. If this quantity is denoted X , it follows from Eq. 8.2 of Mahler (1979) that

$$X = p \cdot \Delta\{O_2\} - \Delta\{PC\}_0, \quad (18.1)$$

TABLE IV
Data Bearing on the Magnitude of Postcontractile Suprabasal ATP Hydrolysis (X) in Frog Sartorius Muscle after a Tetanus at 20°C

Species	<i>R. pipiens</i>			<i>R. temporaria</i>		
	0.2	0.5	1.0	0.5	1.0	Pooled
Tetanus duration (s)						
$\Delta[PCr]_0/[Cr]_T$ (nmol/ μ mol)	46.4±8.4 (10)*	92.5±7.1 (8)*	130.3±6.5 (14)* 135.9±7.6 (10) 132.7±4.9 (25)	101.4±4.6 (10)		
$\Delta[O_2]/[Cr]_T$ (nmol/ μ mol)	7.40±0.42 (9)‡ 7.75±0.29 (10)§ 7.42±0.46 (12) 7.52±0.23 (33)	15.1±1.2 (11)‡ 19.0±2.0 (13)§ 17.1±1.7 (25)	25.6±1.8 (6)‡	16.2±1.2 (12)		
$\frac{\Delta[PCr]_0/[Cr]_T}{\Delta[O_2]/[Cr]_T}$ (nmol/nmol)	6.17±(1.33) (10.6) [3.66-8.68]	5.43±(0.58) (23.4) [4.23-6.63]	5.18±(0.42) (10.1) [4.25-6.11]	6.15±(0.54) (19.2) [5.01-7.29]		
$\Delta h_{90}/[Cr]_T$ (mJ/ μ mol)						
Explained			5.79±0.23 (7)	4.46±0.16 (8)		
Unexplained			4.63±0.26 (10)	3.45±0.16 (10)		
			1.26±0.35 (16.9)	1.01±0.21 (17.2)		
$X/[Cr]_T$ (nmol/ μ mol)	1.0±8.5 (10.6)	15.2±7.2 (8.5)	28.6±12.6 (8.3)	2.6±8.9 (17.9) 24.1±8.4 (14.2) 13.4±6.1 (32.0)		13.6±4.1 (3) [0.4-26.7]

All quantities have been normalized by total creatine (Cr_T) content. X was calculated via Eqs. 18.1 and 18.2 (see text). Values are given as means \pm SEM (degrees of freedom). Values in brackets are 95% confidence limits. When two or more sets of measurements of $\Delta[PCr]_0/[Cr]_T$ or $\Delta[O_2]/[Cr]_T$ were made for a given tetanus duration, grand means were obtained by pooling all individual values. SEM's in parentheses are approximate. Statistics on $\frac{\Delta[PCr]_0/[Cr]_T}{\Delta[O_2]/[Cr]_T}$ were calculated as in Mahler (1979). Statistics on $\Delta h_{90}/[Cr]_T$ (unexplained) and $X/[Cr]_T$ were calculated as described in Bliss (1967; cf. footnote 3). In calculating the pooled value of $X/[Cr]_T$, individual means were weighted by the numbers of observations (Dixon and Massey, 1969).

* Mahler (1979).

‡ Recalculated from data of Mahler (1979).

§ Direct method.

where p denotes the P/O₂ ratio for oxidative metabolism, which has an expected value of ~6.3 mol/mol (Mahler, 1979; Crow and Kushmerick, 1982). Note that if $X = 0$, $\Delta\{\text{PC}\}_0/\Delta\{\text{O}_2\}$ should match the P/O₂ ratio, and, in general, $\Delta\{\text{PC}\}_0/\Delta\{\text{O}_2\} = p - (X/\Delta\{\text{O}_2\})$, so that the higher the value of X , the lower the value of $\Delta\{\text{PC}\}_0/\Delta\{\text{O}_2\}$. For the four experimental conditions studied, after normalization by $\{\text{C}_T\}$, $\Delta\{\text{PC}\}_0/\Delta\{\text{O}_2\}$ ranged from 5.2 to 6.2 $\mu\text{mol}/\mu\text{mol}$. However, the 95% confidence limits on these values were rather wide (Table IV). Values of X were calculated via Eq. 18.1, assuming a value for p of 6.3 mol PC/mol O₂. The individual estimates were statistically compatible with a rather wide range of values (Table IV), but the pooled, weighted mean for $X/\{\text{C}_T\}$, 9.5 ± 4.8 ($n = 3$) nmol/ μmol , was somewhat more precise.

A similar method of quantifying X , which did not require measurement of O₂, was made possible by measurement of suprabasal recovery heat production (Δh_R), in addition to the PC breakdown and suprabasal heat production (Δh_0) during a contraction. Assuming that by the end of recovery the only net change in the tissue is the oxidation of a certain amount of substrate, the total suprabasal heat production, measured as $\Delta h_0 + \Delta h_R$, should equal $H_{\text{O}_2} \cdot \Delta\{\text{O}_2\}$, where H_{O_2} denotes the average enthalpy per mole of O₂ consumed. Eq. 18.1 can thus be rewritten as

$$X = p \frac{\Delta h_0 + \Delta h_R}{H_{\text{O}_2}} - \Delta\{\text{PC}\}_0. \quad (18.2)$$

For a 0.5-s tetanus in the sartorius of *R. temporaria*, in addition to the results listed in Table IV, $\Delta h_R/\{\text{C}_T\}$ was found to be 5.05 ± 0.41 ($n = 8$) mJ/ μmol , and $(\Delta h_0 + \Delta h_R)/\{\text{C}_T\}$ was 9.51 ± 0.53 ($n = 8$) mJ/ μmol . Assuming that $H_{\text{O}_2} = 477$ kJ/mol (Curtin and Woledge, 1978) and $p = 6.3$ mol/mol, $X/\{\text{C}_T\}$ was 24.1 ± 8.4 ($n = 14.2$) nmol/ μmol .⁵ This was not significantly different from the value obtained via Eq. 18.1, 2.6 ± 8.9 ($n = 17.9$) nmol/ μmol , which again illustrates the difficulty of quantifying a small difference between two means. Pooling all estimates of $X/\{\text{C}_T\}$ gave a value of 13.6 ± 4.1 ($n = 3$) nmol/ μmol (Table IV). Assuming a typical value for $\{\text{C}_T\}$ of 35 $\mu\text{mol}/\text{g}$, this is equivalent to a suprabasal ATP hydrolysis of 0.48 ± 0.14 $\mu\text{mol}/\text{g}$ during recovery.

DISCUSSION

Value of D

The revised values of D given here are consistent with those recently reported for hamster skeletal muscles ($1.2\text{--}2.6 \times 10^{-5}$ cm²/s at 37°C) by Ellsworth and Pittman (1984), who used the same methodology, including the hemi-elliptical-cylinder model (Mahler, 1981). No other directly determined values for D_{O_2} in intact muscle appear to have been published. In view of the obvious heterogeneity of muscle tissue, it can be expected that this diffusion coefficient for oxygen is a

⁵ For sample means X and Y with unequal variances V_X and V_Y and degrees of freedom n_X and n_Y , the number of degrees of freedom associated with $X \pm Y$ was calculated as $(V_X + V_Y)^2 / [(V_X^2/n_X) + (V_Y^2/n_Y)]$ (Bliss, 1967), which typically yields noninteger values.

volume-averaged parameter. Its computation is based on the demonstration that in a resting muscle, both the steady state P_{O_2} profile within the tissue (Gore and Whalen, 1968) and the time course of P_{O_2} at its surface are well fit by the appropriate solutions of the diffusion equation for a homogenous hemi-elliptical cylinder whose configuration closely matches that of the muscle (Mahler, 1978*a*, 1985*a*; Ellsworth and Pittman, 1984). These values of P_{O_2} are volume-averaged by the oxygen electrode used to measure them; thus, the calculated values of D (and Q) are of necessity also volume-averaged. This raises the question of the extent to which a measured P_{O_2} profile or time course has been smoothed by the measuring device. (It can be intuitively expected that when a P_{O_2} profile is changing with time, oscillations or other irregularities in the profile will lead to irregularities in the time course of P_{O_2} at a given point.) The ability to detect such irregularities depends on both the size of the electrode-sensing surface and the step size, in distance or time, at which the P_{O_2} measurements are made. In the present case, no oscillations were discernible in continuous recordings of P_{O_2} , but because the electrode had a sensing surface of diameter of 25 μm , any variation within this surface area would have been averaged. Gore and Whalen (1968) measured the steady state intramuscular P_{O_2} profile with a recessed-tip microelectrode having a tip diameter of 2 μm , which considerably reduced the volume sampled by the electrode; however, their measurements were made at steps of 71 μm . In any case, the question of the effects of electrode volume-averaging may be rendered moot by a recent observation of Tsacopoulos et al. (1981). To complement measurements of P_{O_2} within another heterogeneous tissue, the honeybee drone retina, they analyzed the effect of heterogeneity in tissue Q_{O_2} on the P_{O_2} profile predicted by the diffusion equation. For a sheet in which Q_{O_2} varies sinusoidally with the distance perpendicular to the surface, with a period of 31.5 μm and mean denoted Q_m , the steady state solution of the diffusion equation was practically identical to that for the case when Q_{O_2} was constant at Q_m . This suggests that even if D and α , as well as Q , are not uniform within a tissue, as long as their variations are regular (or random), the P_{O_2} profile determined by diffusion will well approximate that in a homogeneous medium in which D , α , and Q are equal to the volume-averaged values in the tissue.

Effect of Tissue Configuration on Calculated Q_{O_2}

The finding that the values of $\Delta Q_{O_2}(t)$ calculated with the hemi-elliptical cylinder model were smaller than those calculated previously with the sheet model (Mahler, 1978*b*, 1979) was unexpected. For a given $\Delta Q_{O_2}(t)$, the drop in P_{O_2} at the center of a hemi-elliptical cylinder [i.e., $P(0, 0, t)$; Fig. 3*A*] will be smaller than that at the bottom of a sheet of equal thickness, since, in the former case, diffusion of O_2 to the point in question occurs in the x as well as in the y direction. Thus, to produce any measured $P(0, 0, t)$, a larger $\Delta Q_{O_2}(t)$ is required in a hemi-elliptical cylinder than in a sheet. Operationally, this effect of tissue geometry per se on the calculated $\Delta Q_{O_2}(t)$ is due to the form of the transfer function $H(x_0, y_0, s)$ in the computational algorithm (Eq. 4). The present finding is explained by the fact that the sheet model was used to determine not only the form of the transfer function, but also the value of D used in its evaluation (Mahler, 1978*a*).

As discussed above, this caused D to be overestimated. This in turn caused $\Delta Q_{O_2}(t)$ to be overestimated: for a given $\Delta Q_{O_2}(t)$, the larger the value of D , the smaller will be the drop in P_{O_2} anywhere in the tissue; conversely, for a given $\Delta P(0, 0, t)$, the larger the value of D , the larger will be the calculated $\Delta Q_{O_2}(t)$. Thus, the use of the sheet model caused two errors in the calculated $\Delta Q_{O_2}(t)$, of opposite sign, with the net result exceeding that calculated with the elliptical cylinder model.

The use of the elliptical cylinder model did not alter the earlier conclusion (Mahler, 1978*b*) that the descending limb of $\Delta Q_{O_2}(t)$ after a tetanus was very well fit by a monoexponential, whose time constant was independent of tetanus duration over the range 0.1–1.0 s. This suggests that during the monoexponential phase of $\Delta Q_{O_2}(t)$, the reactions controlling respiration may be rate-limited by a single step with apparent first-order kinetics. The possible mechanisms underlying the remarkably simple kinetics of muscle Q_{O_2} are investigated in the following paper (Mahler, 1985*b*).

Solubility of O₂ in Muscle

The solubility of O₂ in intact muscle does not appear to have been measured previously. The present result, $\alpha(\text{muscle})/\alpha(\text{H}_2\text{O}) = 1.26 \pm 0.04$, is consistent with the results of Campos Carles et al. (1975) on the solubilities of seven inert gases in rat skeletal muscle, for which $\alpha(\text{muscle})/\alpha(\text{H}_2\text{O})$ ranged from 1.1 to 2.6. The highest values were for gases that were highly lipid-soluble, and for the four gases whose lipid solubilities were similar to that of O₂, $\alpha(\text{muscle})/\alpha(\text{H}_2\text{O})$ was 1.1–1.2. [For O₂, $\alpha(\text{olive oil})/\alpha(\text{H}_2\text{O}) = 3.9$ at 22°C (Battino et al., 1968; Bartels et al., 1971).] It is of interest that Tsacopoulos et al. (1981) recently reported the solubility of O₂ in the drone retina to be 1.8 times that in water.

Hill (1966) estimated that $\alpha(\text{muscle})/\alpha(\text{H}_2\text{O})$ for O₂ in frog muscle was 0.82, by assuming that 97% of muscle water is “free,” dissolving O₂ as does normal water, and that the solubility of O₂ in the rest of the tissue is negligible. However, O₂ can also be expected to dissolve in both the lipid and protein fractions of muscle. We are aware of no published values for the volume fraction of frog muscle occupied by lipid, but Campos Carles et al. (1975) found this to be ~0.04 in rat abdominal muscle, and, as noted above, the solubility of O₂ in olive oil, presumably a representative lipid, is about four times that in water. As for the solubility of O₂ in protein, Stoddard (1927) reasoned on the basis of rather indirect evidence that the solubility of N₂ in the protein fraction of a solution of plasma proteins was negligible. This conclusion was cited by Takahashi et al. (1966), who, like A. V. Hill (1966), assumed that O₂ was excluded from the entire volume occupied by tissue proteins. However, Sendroy et al. (1934), using hemoglobin (Hb) in which oxygen binding was blocked, had shown that the solubility of O₂ in Hb at 38°C, when expressed per cubic centimeter of Hb, was ~1.6 times that in water. A similar value was recently estimated by Weber et al. (1981) with a radically different technique, based on the quenching of protein fluorescence by O₂. This phenomenon has been used to demonstrate the diffusibility of O₂ within a wide variety of proteins (Lakowicz and Weber, 1973*a, b*; Eftink and Jameson, 1982; Lakowicz and Maliwal, 1983). The measured solubil-

ity of O_2 in muscle does not appear to be much different from the sum of the solubilities of O_2 in water, protein, and lipid, weighted by their volume fractions in the tissue. Assuming that these three components account for 78, 20, and 2%, respectively, of the wet weight of a muscle, and that the average densities of protein and lipid are 1.35 and 0.94, respectively (Langerman, 1972; Geyer, 1972), the volume fractions occupied by water, protein, and lipid would be 0.822, 0.156, and 0.022. If the average solubilities of O_2 in protein and lipid are 1.6 and 3.9 times that in water, $\alpha(\text{muscle})/\alpha(\text{H}_2\text{O})$ would be 1.16, compared with the observed value of 1.26 ± 0.04 . It is also possible that at least some intracellular water, because of reduced mobility caused by association with proteins, might dissolve more O_2 than bulk water (see discussion in Mahler, 1978a).

The comparison of the results of the direct and diffusion methods for measuring Q_{O_2} (Table III) provided a check on the accuracy of the measured value of α . All other aspects of the diffusion method (i.e., the description of the tissue boundary, the accuracy of the general form of the solutions of the diffusion equation, and the value of D) had previously been quantified or validated. The good agreement between the results of the two methods thus indicates that the value of α used was substantially correct. Equivalently, these experiments themselves could be considered to provide an indirect determination of α . If α is left unspecified, the diffusion method yields Q_{O_2}/α , while the direct method provides Q_{O_2} , and α can thus be calculated as the value required for the results of the two methods to match. From the measurements of Q_0 , ΔQ_{ss} , and $\Delta\{O_2\}$, normalized by $\{C_T\}$ (Table III), the values of α determined in this way for blotted muscle at 20°C were 0.0365, 0.0389, and 0.0402 ml/cm³·atm, respectively. $\alpha(\text{muscle})/\alpha(\text{H}_2\text{O})$ had a mean of 1.24, which is in excellent agreement with the directly determined value.

The measurement of α , together with the revised values for D , allowed the calculation, as $K = D\alpha$, of the Krogh permeation constant for O_2 in the frog sartorius at 22.8°C . The resulting value, $(2.37 \pm 0.12) \times 10^{-5}$ ml O_2 /cm·min·atm, is higher than the values reported for frog muscle by Krogh (1919) and Gore and Whalen (1968) (1.5 and 1.4×10^{-5} ml O_2 /cm·min·atm, respectively), but appears to be consistent with the values reported recently for rat and chicken muscle [(2.2 ± 0.2) and $(2.3 \pm 0.3) \times 10^{-5}$ ml O_2 /cm·min·atm, respectively, at 37°C ; see Table II] by Kawashiro et al. (1975) and DeKoning et al. (1981). The values of α listed in Table II for temperatures other than 22.8°C were calculated on the assumption that $\alpha(\text{muscle})/\alpha(\text{H}_2\text{O})$ is independent of temperature. Because $\alpha(\text{muscle})$ appears to be determined primarily by $\alpha(\text{H}_2\text{O})$, as just discussed, this is probably substantially correct. However, the solubility of O_2 in lipid appears to be practically constant over the temperature range 25 – 55°C (Battino et al., 1968), while the temperature dependence of the solubility of O_2 in protein is not known, so $\alpha(\text{muscle})/\alpha(\text{H}_2\text{O})$ might vary slightly with temperature.

Comparison of Direct and Diffusion Methods

The direct method required measurements of relatively small changes, or rates of change, in chamber P_{O_2} . Suprabasal recovery O_2 consumption after a 0.2-s

tetanus at 20°C caused a P_{O_2} drop of ~10 mmHg, which is ~2% of the chamber content when it was initially equilibrated with 60.5% O₂, and this change took ~20 min to be completed. For a train of eight twitches per minute, the linear changes in chamber P_{O_2} before and during the series of contractions averaged ~0.8 and 2.6 mmHg/min, respectively, which is ~0.2%/min and ~0.6%/min of the chamber content. However, when the noise level of the oxygen electrode was reduced by the use of multiple membranes (see Materials and Methods), these quantities could be measured precisely (Fig. 7, A and B).

In contrast, the changes in P_{O_2} measured in the diffusion-method experiments were very much larger. The steady state drop in P_{O_2} from the external medium to the center of the closed, lower surface of a resting muscle (ΔP_0) averaged ~130 mmHg, and when the muscle twitched eight times per minute, the further drop in steady state P_{O_2} at this point (ΔP_1) averaged 410 mmHg (Fig. 7C). After a 0.2-s tetanus in the sartorius of *R. pipiens*, the peak drop in P_{O_2} at the lower surface of the muscle had a mean of ~100 mmHg, and after a 0.5-s tetanus in the sartorius of *R. temporaria*, it was 170 mmHg (Fig. 3A).

Energy Balance and Postcontractile ATP Hydrolysis

In studies with frog skeletal muscle, done primarily at 0°C, the energy liberation during a single contraction has not been accounted for by the observed changes in the levels of tissue metabolites. Typically, the only statistically or energetically significant measured change has been the hydrolysis of PC. Using the molar enthalpy for this process given by Curtin and Woledge (1978), -34 kJ/mol, for isometric tetani of 2–15 s at 0°C an enthalpy of ~1.5–3.5 mJ/ μ mol C_T (which amounts to ~20–60% of the total energy liberation) has remained unexplained (Curtin and Woledge, 1978, 1979; Homsher and Kean, 1978; Homsher et al., 1979). The present results (Table IV) show that this also occurs for brief tetani in the sartorii of both *R. pipiens* and *R. temporaria* at 20°C.

It is natural to speculate that the processes causing the unexplained enthalpy during a contraction are reversed during recovery, at the expense of suprabasal ATP hydrolysis (Curtin and Woledge, 1978; Homsher and Kean, 1978). Attempts to demonstrate postcontractile suprabasal ATP hydrolysis directly have largely been negative (cf. discussions in Curtin and Woledge, 1978; Mahler, 1979), but indirect methods have strongly suggested that it does occur to an appreciable extent in the sartorius of *R. pipiens* in both well-oxygenated muscles at 0°C (Kushmerick and Paul, 1976b) and anaerobic muscles at 20°C (DeFuria and Kushmerick, 1977). The present results point to a small postcontractile suprabasal ATP hydrolysis, ~10–20 nmol/ μ mol C_T, in well-oxygenated sartorii of both *R. pipiens* and *R. temporaria* at 20°C. This value appears to be comparable to the ATP cost of postcontractile calcium pumping. Somlyo et al. (1982) found that after a tetanus of 1.2 s in the frog semitendinosus muscle at 22°C, beginning 0.4 s after the cessation of stimulation, just after the muscle had relaxed (the same time at which muscles were frozen in the present experiments for measurements of $\Delta\{PC\}_0$), the amount of Ca⁺⁺ returned to the terminal cisternae (TC) of the sarcoplasmic reticulum was 38 ± 8.5 ($n = 122$) μ mol/g dry TC. Assuming that the TC have a water content of 72% by weight and account for 4.5% of cell

volume (Somlyo et al., 1982), and that 1 ATP is hydrolyzed for every 2 Ca^{++} reaching the TC (Tada et al., 1978), the calculated ATP splitting associated with the observed calcium movement is $0.24 \pm 0.05 \mu\text{mol/g}$, or $\sim 7 \text{ nmol}/\mu\text{mol C}_T$.

APPENDIX

Predicted Time Course of Chamber P_{O_2} for the Direct Method with a Sheet-like Tissue

For a sheet of tissue in a closed, well-stirred chamber, if the diffusion equation for O_2 is valid on the macroscale in the tissue, the time course of chamber P_{O_2} , $P(y, t)$, solves

$$D\alpha_m \frac{\partial^2 P}{\partial y^2}(y, t) - \alpha_m \frac{\partial P}{\partial t}(y, t) = Q(t), \quad (\text{A1.1})$$

$$lv \frac{\partial P}{\partial t}(l, t) + D \frac{\partial P}{\partial y}(l, t) = 0, \quad (\text{A1.2})$$

$$\frac{\partial P}{\partial y}(0, t) = 0, \quad (\text{A1.3})$$

$$0 \leq y \leq l, \quad (\text{A1.4})$$

where $2l$ is the tissue thickness, D and α_m are the diffusion coefficient and solubility of O_2 in the tissue, the parameter v is equal to $\alpha_R V_R / \alpha_m V_m$, α_R is the solubility of O_2 in the bath, and V_R and V_m are the volumes of the bath and the muscle. The boundary condition A1.2 expresses the equality between the rate of O_2 decrease in the bath and the flux of O_2 into the tissue. The boundary condition A1.3 is a symmetry condition at the center of the muscle. For the case $v = \infty$, the boundary condition A1.2 becomes $P(l, t) = P_0$, which is the boundary condition for the diffusion method.

Louy (1983) has derived the solution of Eqs. A1.1–A1.4. We give the solution here for the particular case in which the tissue has, at $t = 0$, the steady state profile

$$P(y, 0) = P_1 - (Q_0 l^2 / 2D\alpha_m)[1 - (y/l)^2], \quad (\text{A2})$$

for which the bath P_{O_2} is constant at P_1 , and the Q_{O_2} is at its basal value (Q_0). The time course of the chamber P_{O_2} for the system of Eqs. A1.1–A1.4 then can be written as

$$P(t) = P_1 - Q_0 l^2 / 3D\alpha_m (1 + v)^2 \quad (\text{A3, term 1})$$

$$+ (2Q_0 l^2 / D\alpha_m) \sum_{n=1}^{\infty} \frac{e^{-(\mu_n^2 D/l^2)t}}{\mu_n^2 (\mu_n^2 V^2 + v + 1)} \quad (\text{term 2})$$

$$- (wQ_0 / \alpha_m)t \quad (\text{term 3})$$

$$- (w/\alpha_m) \int_0^t \Delta Q(z) dz \quad (\text{term 4})$$

$$+ (2v/\alpha_m) \int_0^t \Delta Q(z) \sum_{n=1}^{\infty} \frac{e^{-(\mu_n^2 D/l^2)(t-z)}}{\mu_n^2 v^2 + v + 1} dz. \quad (\text{term 5})$$

In this equation, P_1 is the chamber P_{O_2} at time $t = 0$, $\Delta Q(t)$ is the time course of suprabasal Q_{O_2} , the term w designates $1/(1 + v)$, and $\{\mu_n\}$ are the roots of the transcendental equation $\tan \mu_n = -v\mu_n$. For further details, consult Louy (1983).

Validation of Eq. 7.4 for Respiring Tissue

From this solution, one can obtain the solution for the protocol used to measure α_m . In that case, the tissue also has an initial steady state P_{O_2} profile, but with boundary value P_0 , and at $t = 0$ the boundary value is changed to P_1 . $\Delta Q(t)$ is zero. This situation can be described in terms of the general case treated above, by letting $\Delta Q(t)$ be an impulse function centered at $t = 0$. This will cause the P_{O_2} everywhere in the tissue to drop by a fixed amount at $t = 0$, while the P_{O_2} of the bath remains at P_1 . If the impulse has magnitude $\alpha_m(P_1 - P_0)$, the drop will be $P_1 - P_0$, and the effect will be the same as if the boundary P_{O_2} of a resting muscle were raised from P_0 to P_1 . Thus, for the case $\Delta Q(t) = \alpha_m(P_1 - P_0)\delta(t)$, where $\delta(t)$ denotes the unit impulse at $t = 0$, Eq. A3 gives the predicted time course of chamber P_{O_2} for the protocol used to measure α_m .

We want to calculate the intercept at $t = 0$ of the final linear phase of this predicted $P(t)$; this is the quantity Δ determined experimentally (Fig. 1C). Term 1 of Eq. A3 is a step decrease of magnitude

$$\Delta_1 = Q_0 l^2 / 3D\alpha_m [1 + (\alpha_R V_R / \alpha_m V_m)]^2. \quad (A4)$$

Terms 2 and 5 vanish for large t , and so contribute nothing to the intercept. Term 3 is linear, with zero intercept. Term 4 is a step decrease of magnitude

$$\Delta_4 = w(P_1 - P_0) = (P_1 - P_0) / [1 + (\alpha_R V_R / \alpha_m V_m)]. \quad (A5)$$

It follows that $\Delta = \Delta_1 + \Delta_4$, while Eq. 7.4 assumes $\Delta = \Delta_4$. Δ_1 is a correction term that takes into account the change in the form of the P_{O_2} profile of the tissue between $t = 0$ (Eq. A2) and the end of the experiment (Eq. A3). This term did not appear in Eq. 7.4 because it was assumed that $Q_0 = 0$. However, given that the muscle volume is very small compared with the bath volume, Δ_1 can be expected to be small. In fact, under the present conditions ($Q_0 \approx 0.7 \mu\text{l/g}\cdot\text{min}$; $l \approx 0.045 \text{ cm}$; $D \approx 1 \times 10^{-5} \text{ cm}^2/\text{s}$; $\alpha_m \approx 0.037 \text{ ml O}_2/\text{cm}^3\cdot\text{atm}$; $\alpha_R V_R / \alpha_m V_m \approx 40$; $P_1 \approx 699 \text{ mmHg}$; $P_0 \approx 155 \text{ mmHg}$), Δ_1 was typically only 0.01 mmHg, while Δ_4 was $\sim 15 \text{ mmHg}$. This justified the assumption that $\Delta \approx \Delta_4$ made by Eq. 7.4, and the use of Eq. 7.3 to calculate α_m .

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