ANNALS OF BIOMEDICAL ENGINEERING 5, 260–272 (1977)

Oxygen and Lactic Acid Transport in Skeletal Muscle

Effect of Reactive Hyperemia

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Received November 7, 1976

A mathematical model of oxygen and lactic acid transport in skeletal muscle is used to test the effects of reactive hyperemia on oxygen and lactic acid concentrations following a period of ischemia. The model is based on the Krogh cylinder as the geometrical representation of the functional unit of transport, i.e., a capillary and the tissue it supplies. Included in the mathematical development of the model are the convective and diffusive transport of the chemical species, the nonlinear aspects of oxygen and lactic acid kinetics, and the reversible reaction of oxygen with hemoglobin in capillary blood and myoglobin in the tissue. The steady-state solution to the model is obtained first as the baseline for the study. Ischemia is then simulated by the cessation of capillary blood flow. This is followed by a reactive hyperemic response that is a function of the occlusion duration. The general effect of reactive hyperemia is to shorten the time intervals for initial return of tissue oxygen levels and the washout of accumulated lactic acid and to maintain tissue oxygen levels above steady-state values.

INTRODUCTION

Krogh (1918–1919, 1922) proposed that the regular arrangement of capillaries in skeletal muscle could be utilized to develop the now familiar Krogh cylinder as the geometrical unit for modeling the transport of oxygen to skeletal muscle. Since that time many theoretical investigations have been undertaken on the transport of oxygen to living tissues. Detailed historical reviews are available (Middleman, 1972; Lightfoot, 1974).

In the present paper several aspects of transient effects in such a model that have not been previously reported are considered. The first of these is the role of reactive hyperemia. This phenomenon is characterized by an excess blood flow following the release of occlusion. It has been studied in whole organs (Lewis and Grant, 1925; Goldblatt, 1926; Hyman *et al.*, 1963) and at the capillary level (Johnson and Wayland, 1967; Burton and Johnson, 1972; Gentry and Johnson, 1972). The cause of this response is not exactly known, but there are two current hypotheses based on either metabolic or myogenic mechanisms (Johnson and Wayland, 1967).

Important to the modeling process is the mathematical characterization of this phenomenon. Plethysmography studies (Hyman and Wong, 1968) indicate that

ISSN 0090-6964

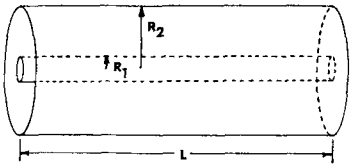

FIG. 1. Krogh cylinder.

a decreasing exponential with a decay constant that is a function of the occlusion period aptly describes the hyperemic response.

Other recent investigators of the microcirculation (Johnson and Wayland, 1967; Burton and Johnson, 1972; Gentry and Johnson, 1972) have characterized the magnitude of the response by the area under the hyperemic curve above the control flow. They also define "flow debt" as the time of occlusion times the average flow, enabling one to describe the response as a flow debt repayment (FDR). They also found that within limits, the FDR was approximately three times the flow debt. The post-occlusion peak capillary flow averaged about 200% of the resting value. It was also noted that approximately 4 sec were required for a peak flow to be reached following the release of an occlusion. Based on these data, an empirical flow scheme has been devised to describe the hyperemic response as a function of the occlusion duration.

In addition to oxygen transport and utilization, the present model includes anaerobic metabolism with lactic acid production and its diffusion.

These new features of reactive hyperemia and anaerobic metabolism are incorporated into the basic approach utilized by Hyman *et al.* (1975) to study transient effects with a simplified "oxygen dept" concept and by Artigue and Hyman (1976) to study the role of myoglobin in the space–time distribution of oxygen in skeletal muscle subjected to ischemia.

Using the Krogh cylinder as shown in Fig. 1, a mass balance is performed in the usual way on an elemental volume which extends across the entire capillary. The rate of change of total concentration is set equal to the outward flux through the capillary wall.

For oxygen the capillary equation becomes

$$\frac{\partial C_b}{\partial t} + N\frac{\partial \Psi_1}{\partial t} = -V\frac{\partial C_b}{\partial z} - VN\frac{\partial \Psi_1}{\partial z} - \frac{2J}{R_1}, \tag{1}$$

where C_b is the concentration of oxygen in the blood (cm^3 O_2/cm^3 blood), t is time (sec), z is the axial coordinate (cm), R_1 is the radius of the capillary (cm), V is the average capillary blood velocity (cm/sec), N is the oxygen capacity of blood, (cm^3 O_2/cm^3 blood), Ψ_1 is the fractional saturation of oxygen in blood, and J is the flux out through the capillary wall (cm^3 O_2/cm^2 sec). The total blood flow rate is obtained by multiplying the velocity V by the cross-sectional area πR_1^2.

In terms of the partial pressure of oxygen the equation is

$$\frac{\partial p_b}{\partial t} = -V\frac{\partial p_b}{\partial z} - \frac{2J}{R_1[S_b + N(\partial \Psi_1/\partial p_b)]}, \tag{2}$$

where p_b is the partial pressure of oxygen in the blood (mm Hg) and S_b is the solubility of oxygen in blood (cm^3 O_2/cm^3 blood mm Hg). In terms of the partial pressure Ψ_1 is given by

$$\Psi_1 = K_1 p_b^n/(1 + K_1 p_b^n). \tag{3}$$

In this relation K_1 and n are constants associated with temperature and pH. The term J is related to conditions in the tissue by

$$J = -DS_t[\partial p_t/\partial r]_{r=R_1}, \tag{4}$$

in which D is the diffusion coefficient of oxygen in the tissue (cm^2/sec), S_t is the solubility of oxygen in the tissue (cm^3 O_2/cm^3 tissue mm Hg), p_t is the partial pressure of oxygen in the tissue (mm Hg), and r is the radial coordinate (cm).

For lactic acid the capillary mass balance leads to

$$\frac{\partial C^*_b}{\partial t} = -V\frac{\partial C^*_b}{\partial z} - \frac{2J^*}{R_1}, \tag{5}$$

where C^*_b is the concentration of lactic acid in the blood (μmole/cm^3) and

$$J^* = -D^*[\partial C^*_t/\partial r]_{r=R_1} \tag{6}$$

is the flux of lactic acid through the capillary wall (μmole/cm^2 sec). Here D^* is the diffusion coefficient of lactic acid in the tissue (cm^2/sec) and C^*_t is the concentration of lactic acid in the tissue (μmole/cm^3).

Implicitly contained in these equations is the assumption that the capillary blood is homogeneous with the source–sink role of the erythrocytes assumed to be uniformly distributed. The average capillary velocity is used as a plug flow velocity and details with respect to the radial direction are not considered. It is also assumed that there is an instantaneous release and binding of oxygen to hemoglobin. Not explicitly considered in these simulations are the effects of many of the known vasoactive substances, such as histamine and serotonin, whose release or presence in the blood might change the capillary velocity.

In developing the differential equations for the tissue a similar mass balance is performed by setting the total rate of change of concentration equal to the difference between the net flux due to diffusion and the mass removed or gained by chemical reaction.

For oxygen in the presence of myoglobin the tissue equation becomes

$$\frac{\partial C_t}{\partial t} + Q\frac{\partial \Psi_2}{\partial t} = D\left(\frac{\partial^2 C_t}{\partial r^2} + \frac{1}{r}\frac{\partial C_t}{\partial r} + \frac{\partial^2 C_t}{\partial z^2}\right) - M. \tag{7}$$

Here M represents the oxygen consumption rate in the tissue (cm^3 O_2/cm^3 tissue sec), Q is the oxygen binding capacity of the tissue due to myoglobin (cm^3 O_2/cm^3 tissue), and Ψ_2 is the percentage saturation of myoglobin with oxygen which is defined by the equation

$$\Psi_2 = K_2 p_t/(1 + K_2 p_t), \tag{8}$$

where K_2 is a constant. The assumptions and justification for this description of the function of myoglobin are given in Artigue and Hyman (1976).

In terms of partial pressure this equation is

$$\left(1 + \frac{Q}{S_t}\frac{\partial \Psi_2}{\partial p_t}\right)\frac{\partial p_t}{\partial t} = D\left(\frac{\partial^2 p_t}{\partial r^2} + \frac{1}{r}\frac{\partial p_t}{\partial r} + \frac{\partial^2 p_t}{\partial z^2}\right) - \frac{M}{S_t}. \tag{9}$$

For lactic acid the tissue equation is

$$\frac{\partial C^*_t}{\partial t} = D^*\left(\frac{\partial^2 C^*_t}{\partial r^2} + \frac{1}{r}\frac{\partial C^*_t}{\partial r} + \frac{\partial^2 C^*_t}{\partial z^2}\right) + A - L, \tag{10}$$

where the term A is the production rate of lactic acid (μmole/cm^3 sec) and L is its oxidation rate (μmole/cm^3 sec).

To obtain a steady-state solution to the model V is set equal to V_B, the basal capillary velocity which is assumed to be constant. Having this solution, ischemia is simulated by setting V equal to zero for the prescribed occlusion period. At the end of this period, reactive hyperemia is simulated by an empirical expression in which V becomes

$$V = V_{max}(1 - e^{-\delta_1 t}) \tag{11}$$

for $t \leq t^*$, where $t = 0$ begins the recovery period, t^* is the time at which the peak flow occurs, and V_{max} is the maximum velocity occurring following any occlusion period. For $t > t^*$ the velocity is described by

$$V = V_B(1 + e^{-\delta_2(t-t^*)}). \tag{12}$$

These forms are consistent with the observations of Burton and Johnson (1972), and Gentry and Johnson (1972).

The rise coefficient δ_2 is a function of the occlusion period and the prescribed flow debt repayment (FDR). This coefficient is determined from

$$\text{FDR} = \int_0^\infty (V - V_B)dt \times 100 \Big/ V_B \times \text{period of occlusion}, \tag{13}$$

which leads to an integral equation for δ_2 which can be solved numerically. The capillary velocities for a 100 and 200% FDR are shown in Fig. 2.

The boundary conditions for the capillary–tissue system are described as follows:

Capillary–tissue interface, $r = R_1$. For oxygen the partial pressure in blood is equal to the partial pressure in the tissue or

$$[p_b]_{R_1} = [p_t]_{R_1}. \tag{14}$$

For lactic acid the concentration in the blood is equal to the partition coefficient λ times the concentration in the tissue, or

$$[C^*_b]_{R_1} = \lambda[C^*_t]_{R_1}. \tag{15}$$

Outer boundaries of tissue cylinder. For oxygen the partial pressure gradients

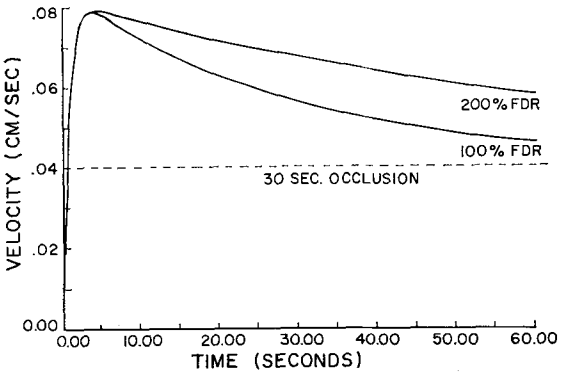

FIG. 2. Capillary velocity following a 30-sec occlusion.

at the outside radius of the tissue and at both ends of the cylinder are equal to zero, or

$$[\partial p_t/\partial r]_{R_2} = [\partial p_t/\partial z]_{z=0,L} = 0 \tag{16}$$

For lactic acid the concentration gradients are also equal to zero at those boundaries, such that,

$$[\partial C^*_t/\partial r]_{R_2} = [\partial C^*_t/\partial z]_{z=0,L} = 0. \tag{17}$$

An integral part of the present study is the development of the metabolic scheme. This scheme incorporates several independently studied phenomena into an internally consistent set of rate expressions through the introduction of a unifying assumption about local energy demand. Without the use of an energy demand criterion, it is difficult to relate aerobic and anaerobic metabolic rates. However, even with this criterion it is not possible to arrive at a set of descriptive equations with which there would be universal agreement. The best that can be accomplished at the present time is the integration of a variety of biochemical and physiological studies within the framework of a set of assumptions which are reasonable in terms of the available literature. Alternative metabolic schemes and/or different numerical values of key parameters could be incorporated into the methodology of this study but these possibilities have not been pursued.

The assumptions used in the present study were:

(1) The energy requirement of muscle tissue at basal conditions is constant and remains so during capillary occlusion and the following recovery period.

(2) Tissue energetics are based on glucose degradation and can be completely described by the consumption of oxygen and the production and oxidation of lactic acid.

(3) Oxidative and anaerobic *energy* production rates are functions of only the oxygen concentration.

Assumption 1 is based on experimental observations which have determined that the oxygen consumption rates in skeletal muscle are nearly constant (Stainsby and Otis, 1964; Folkow and Holicka, 1968). These studies imply that there is a

constant production of energy due to oxidative processes, at least at the basal conditions considered in the experiments. The extension of these results to periods of occlusion and recovery is logically made since the energy produced under basal conditions is felt to be the minimum energy production level which is compatible with normal physiology.

Assumption 2 requires that the energy produced by the cells be derivable from the breakdown of glucose via anaerobic or aerobic glycolysis and the oxidation of pyruvate produced by glycolysis or directly from lactic acid. While Keul *et al.* (1972) have shown that the energy supply in skeletal muscle can almost be completely described by the combination of the degradation of glucose, glycogen, and free fatty acid, in order to make the present model amenable to mathematical analysis only glucose degradation was considered.

Assumption 3 is an oversimplification of actual cellular metabolism. Enormously complicated interactions of substrates and enzymes are involved in an actual system (Lehninger, 1970). The rate-limiting steps of glycolysis and oxidative processes are manyfold including, for example, the concentrations of ADP, ATP, substrates, or various enzymes (Jobsis, 1964; Krebs, 1970). The assumption as used reflects the dependence of cellular metabolism on the presence of oxygen, which is required for the efficient energy yielding oxidative processes. However, this assumption does not imply that the consumption rate of oxygen is not dependent on lactic acid concentration. As developed in the equations below this dependence is explicitly included and in fact represents part of the local "oxygen debt."

Using available data on basal conditions and our assumptions, we set

$$E_B = \text{constant}, \tag{18}$$

where E_B is the basal ATP production rate. By assumptions 1 and 2

$$E_B = E_{OXD} + E_{ANA}, \tag{19}$$

where E_{OXD} is the ATP production rate due to oxidative processes and E_{ANA} is the ATP production due to anaerobic process, or in this case that which is due to lactic production by glycolysis.

At basal conditions

$$E_{ANA} = 0. \tag{20}$$

Therefore, the basal ATP production rate is given by

$$E_B = \alpha_1 M_B, \tag{21}$$

where α_1 is a proportionality constant and M_B is the basal metabolic oxygen consumption rate which is a constant.

Brin *et al.* (1952) have shown that the percentage of the oxygen consumption due to lactic acid oxidation increases as a function of the lactic acid concentration. From these data a relation was obtained which gives the percentage of oxygen consumption due to lactic acid oxidation (P_L) as a function of the lactic acid

concentration:

$$P_L = \frac{P_{L\,max}[C^* - C_s]}{\Omega + [C^* - C_s]}, \qquad \text{for } C^* > C_s,$$

$$= \qquad 0, \qquad \text{for } C^* \leq C_s. \tag{22}$$

Here $P_{L\,max}$ is the maximum percentage of lactic acid oxidation, Ω is a constant, C^* is the concentration of lactic acid, and C_s is the basal, tissue concentration of lactic acid.

It follows then that

$$P_G = 1 - P_L, \tag{23}$$

where P_G represents the percentage of oxygen consumption due to the breakdown of glucose.

The term E_{OXD} can be broken down further such that

$$E_{OXD} = E_G + E_{LA}, \tag{24}$$

where E_G is the energy production rate due to the oxidation of glucose and E_{LA} is that rate due to the oxidation of lactic acid.

It is then easy to describe E_{OXD} as

$$E_{OXD} = (P_G\alpha_1 + P_L\alpha_2)M, \tag{25}$$

where M is the oxygen consumption rate for all concentrations of oxygen and lactic acid and α_2 is the proportionality constant which relates lactic acid oxidation to ATP production.

But by assumption 3 and since oxidative processes are limited by the concentration of oxygen (Chance, 1957; Jobsis, 1964) E_{OXD} can be written in a Michaelis–Menten-type relation,

$$E_{OXD} = \alpha_1 M_B pO_2/(K^1 + pO_2), \tag{26}$$

where K^1 is a constant and pO_2 is the partial pressure of oxygen.

Therefore, by equating Eqs. (25) and (26), the oxygen consumption rate can be determined such that

$$M = P_{BM}M^1, \tag{27}$$

where P_{BM} is defined as

$$P_{BM} = \alpha_1/(P_G\alpha_1 + P_L\alpha_2), \tag{28}$$

and M^1 as

$$M^1 = M_B pO_2/(K^1 + pO_2). \tag{29}$$

Included in Eq. (27) is what may be called the metabolic oxygen debt, which is incurred when the concentration of lactic acid is above normal levels, and oxygen is available. Part of the increased oxygen delivery observed in post-occlusion periods is also due to the resupply of tissue oxygen levels and myoglobin if present.

From Eq. (19) it is seen that in general

$$E_{ANA} = E_B[1 - (E_{OXD}/E_B)]. \tag{30}$$

Therefore, Eqs. (21), (25), and (27) lead to

$$E_{ANA} = E_B[1 - (M^1/M_B)]. \tag{31}$$

Now since glycolysis produces equal amounts of ATP and lactic acid, Eq. (14) can be used to describe the lactic acid production rate (A) as

$$A = \gamma E_{ANA}. \tag{32}$$

A similar formulation is considered to determine the lactic acid oxidation rate (L) as a function of the lactic acid and oxygen concentrations. The result is

$$L = \alpha_3\alpha_1 M^1(1 - P_G P_{BM}), \tag{33}$$

where α_3 is a proportionality constant. The proportionality constants α_1, α_2, α_3, and γ are all determined from data obtained in isolated biochemical systems (Lehninger, 1970). While the applicability of such data to intact functioning cells has not been established, this is the best information available at the present time.

MODEL PARAMETERS

The determination of appropriate values for the parameters of the model involves many uncertainties. The anatomical dimensions of the tissue cylinder require an estimate of capillary length and tissue radius. Eriksson and Myrhage (1972) have shown that the average capillary length found in skeletal muscle is approximately 1000 μm but that cross anastomoses exist at approximately every 200 μm. From these data a length equivalent to a capillary without side branches is chosen. Tissue radii can be determined from capillary densities (Schmidt-Nielsen and Pennycuik, 1961; Eriksson and Myrhage, 1972; Hammersen, 1968; Hermansen, 1971).

The diffusion coefficient for lactic acid in the tissue was determined by Eggleton *et al.* (1928). They found that this coefficient was a function of the lactic acid concentration with the lowest value occurring at fatigue levels of lactic acid. The mean of their data was determined and used in the model. The average values (Diamant *et al.*, 1968) for blood and muscle tissue lactic acid levels were used to determine the partition coefficient.

It has been shown (Duling and Berne, 1970) that longitudinal oxygen gradients exist in the precapillary vessels, therefore, a value was chosen for the inlet partial pressure of oxygen, pO_2, which is somewhat lower than that used by other investigators (Renéau *et al.*, 1967; Hyman *et al.*, 1975; Whalen and Nair, 1970) but represents the lower limit found in Keul *et al.* (1972).

The simulation of reactive hyperemia requires four parameters. The value of V_{max} was chosen to be twice the basal velocity. The parameter δ_1 was chosen to simulate the delay to peak flow following an occlusion and δ_2 was calculated to complete the description of the flow debt repayment.

NUMERICAL ANALYSIS

The equations which describe the diffusion and reactions of oxygen and lactic acid in the tissue are both second order, nonlinear partial differential equations.

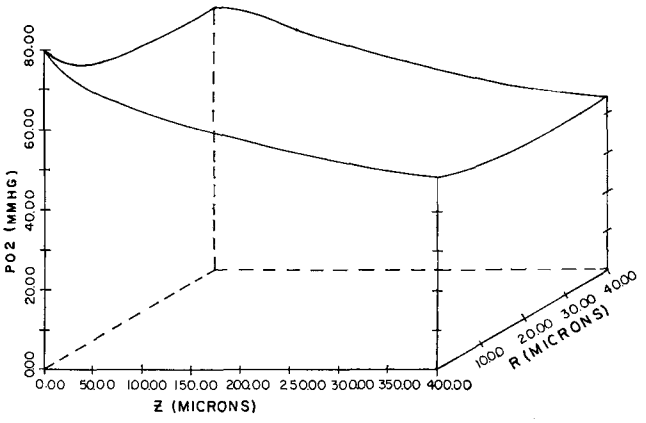

FIG. 3. Steady-state oxygen distribution.

These equations are solved using the implicit alternating direction technique (Peaceman and Rachford, 1955). The equations applicable to the capillary region are first-order, nonlinear partial differential equations. A fully explicit differencing approach is used for these. The equations of the model were programmed on an IBM 360-65 digital computer.

RESULTS

The steady-state distribution of oxygen in the tissue is shown in Fig. 3. The capillary oxygen distribution is included here by the curve for $r = R_1$ (from boundary conditions, Eq. (14)). This result is the same as that reported previously (Artigue and Hyman, 1976) and is included here as reference values for the results to follow. At steady state the lactic acid distribution is uniform in the tissue at 3.0 μmole/cm^3 tissue and in the capillary at 1.4 μmole/cm^3 blood. This is dictated by assumptions in the metabolic scheme. The difference is due to the partition coefficient (Eq. (15)).

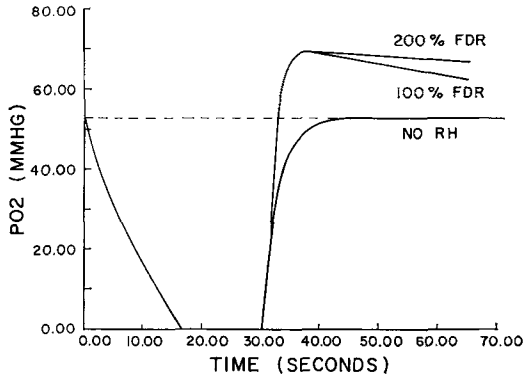

FIG. 4. Oxygen recovery without myoglobin.

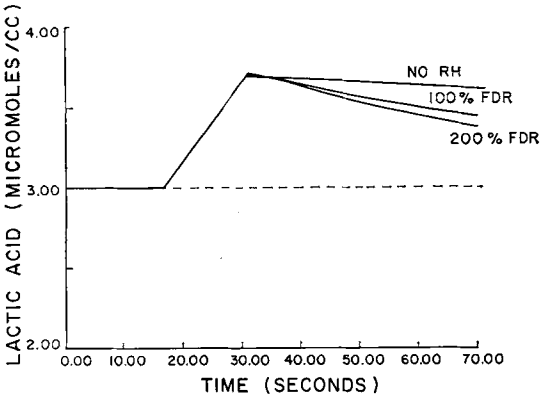

FIG. 5. Lactic acid recovery without myoglobin.

The graphs in Figs. 4–6 show the recovery process following a 30-sec occlusion in the absence of myoglobin for no reactive hyperemia, reactive hyperemia with 100% FDR, and reactive hyperemia with 200% FDR.

From Fig. 4 it can be seen that the time for oxygen to reach the midpoint of the system $[Z = L/2, r = (R_1 + R_2)/2]$ following the resumption of blood flow is approximately 2 sec. It is further seen that without reactive hyperemia the time required to reach normal pO_2 levels is approximately 15 sec from the end of the occlusion period. For both of the reactive hyperemia cases an overshoot is found in which the normal pO_2 is surpassed after 3 sec of recovery. As expected, higher pressures are maintained for a longer period in the case of 200% FDR as shown by the comparison of the decay of the overshoot to normal values in the two hyperemic responses.

The lactic acid concentration response at the LC for the same cases is shown in Fig. 5. As expected the maximum concentration occurs at the end of the occlusion period. The differences seen for the reactive hyperemia cases are small, but the 200% FDR case does indicate that a faster recovery would occur. Without

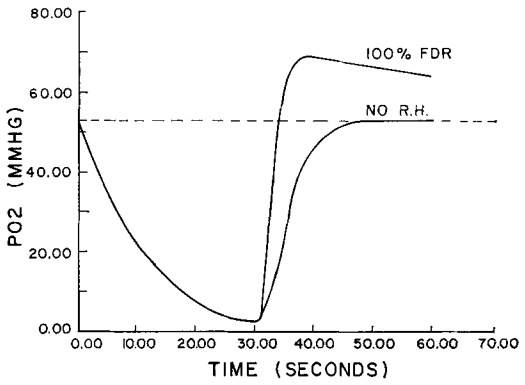

FIG. 6. Oxygen recovery with myoglobin.

the reactive hyperemia the time constant for washout to normal levels is on the order of 12 min and that for 200% FDR 9 min. Although the concentration levels of lactic acid which are involved are, in light of recent findings, probably not significant for this period of occlusion the indication is that high levels of lactic acid could exist for long durations if occlusion is of sufficient duration. This is important since it is known that high levels of lactic acid inhibit glycolysis which would subsequently decrease the ATP production whether or not oxygen is present.

Another case studied was the response in the presence of myoglobin to a 30-sec occlusion and subsequent recovery with and without reactive hyperemia. Again the characteristic overshoot is seen in the recovery with reactive hyperemia of 100% FDR as shown in Fig. 6. For a 30-sec occlusion with myoglobin lactic acid levels do not increase since the oxygen concentration does not fall below 2 mm Hg.

DISCUSSION

The quantitative aspects of the solutions to the model as seen in the previous results are important in themselves, but are more important as tools in understanding the qualitative aspects of this system's response characteristics. As expected, reactive hyperemia decreases the initial recovery time but also involves an overshoot above normal oxygen levels. It is noteworthy that the time required for the lactic acid in the tissue to be removed during recovery is much greater than that necessary for reoxygenation. This suggests that oxygen may not be the key species involved in tissue recovery and the control of related physiological responses. It must be kept in mind, however, that, as in all models, the assumptions leading to the mathematical formulation determine the nature of the results.

In order to extend a study such as this, it is important to determine when the transition occurs from a purely physiological response to a pathological condition. Significant changes in the system can occur during an occlusion which would invalidate several of the assumptions used in developing the present model. For example, cellular breakdown with the concomitant release of cellular constituents would significantly alter the local biochemistry as well as the physical and chemical environment. Therefore, many of the chosen parameters of the model would become invalid. Also neglected is the chemotaxic response elicited by this breakdown with the subsequent alteration of tissue as well as capillary structure.

Another factor heretofore unnoticed, is the possibility of an oxygen excess occurring for long periods in the tissues after periods of occlusion. The overshoots found in the cases with reactive hyperemia are the result of the transient increased capillary flow. Since this response is due to precapillary control, it is clear that if this fine control is lost during an occlusion, a period of sustained excessive flow could result with the subsequent maintenance of an oxygen excess as well as the possible washout of some vital metabolic product needed in the tissue.

To test these as well as other hypotheses developed with the use of this or similar models, increasingly sophisticated experiments performed at the capillary level are required to obtain data on capillary blood flow rate and local hematocrit,

inlet and outlet concentrations of metabolites, and geometrically well-characterized measurements of tissue pO_2.

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