CAPTURE OF SPATIALLY HOMOGENEOUS CHEMICAL REACTIONS IN TISSUE BY FREEZING

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ABSTRACT A useful technique in studying the saturation of hemoglobin in erythrocytes or myoglobin in tissue is cryophotometry, in which tissue is frozen for later spectrophotometric analysis. A general question associated with this technique is whether the freezing process alters the chemical state. This paper presents a theoretical analysis of the simplest model relevant to that question. We study the effect of rapid cooling on a spatially homogeneous chemical reaction. The analysis shows that changes during freezing are negligible near the boundary to which the heat sink is applied, but can be significant deeper in the sample. The distance from the boundary at which the changes during freezing become appreciable can be expressed simply in terms of the chemical reaction rates and the thermal diffusivity of the tissue. Detailed results are given for the case of oxygen and myoglobin in skeletal muscle.

I. INTRODUCTION

The technique of freezing tissue in vivo for later examination by spectrophotometry has proved a valuable tool for capturing the instantaneous chemical state of living tissue. This technique, called cryophotometry, has been used to study hemoglobin saturation in erythrocytes (Grunewald and Lübbers, 1969, 1975, 1976; Acker et al., 1978) and myoglobin saturation in skeletal muscle (Gayeski and Honig, 1978, 1981; Gayeski, 1981). An important question associated with the technique is the following: How much change occurs in the chemical state of the tissue during the freezing process? There is little chance of obtaining an experimental answer to this question, since the ability to do so would obviate the cryophotometric technique itself. A theoretical approach is also very difficult because of the complexity of the events that are to be captured by freezing. However, by using simple models to elucidate mechanisms, we can acquire some insight into the effects of freezing. In this paper, we study the most elementary relevant model, namely, the freezing of a spatially homogeneous state of initial chemical equilibrium. In spite of the simplicity of this model, a surprising amount can be learned from it.

Consider a more detailed description of an example of the kind of problem studied here. Suppose we have red muscle tissue in steady state, with oxygen, myoglobin, and oxymyoglobin in equilibrium. Now we suddenly apply a low temperature heat sink (e.g., a metal block in contact with liquid nitrogen) to one boundary of the tissue. A freezing front will diffuse into the tissue. As the temperature drops, the chemical kinetic coefficients will decrease, but not in the same ratio, and the state of the system will change. Near the boundary, the freezing occurs quickly, and the deviation from the initial state is slight. Far from the boundary, the duration of the freezing process is much longer, and significant changes in the chemical state can occur. One of the principal results of this paper is the determination of the extents of these near and far regions.

Section II gives a general formulation of the problem. In section III, there is a detailed development of the theory for the case of spatially homogeneous reactions. Basic scale relations are given there, as well as specific results for the example of oxygen and myoglobin in tissue. Section IV contains (*a*) some remarks on the more complicated case of spatially inhomogeneous composition, (*b*) a brief discussion of more general thermal configurations than the one analyzed here, (*c*) a discussion of the use of myoglobin as an oxygen indicator in muscle tissue, and (*d*) a summary of this work.

II. GENERAL FORMULATION

Transport Equations

We suppose that we have three components of special interest denoted by M, O, and MO (e.g., myoglobin, oxygen, and oxymyoglobin). The processes that can change the concentrations are diffusion and chemical reactions. For diffusion, we take Fick's law with diffusivities D_M, D_O, and D_{MO}. For the reaction, we take

$$M + O \rightleftarrows MO. \tag{1}$$

We let G be the reaction rate (moles of M transformed to MO per unit volume and time). There may be other reactions involving one of M, O, or MO with other components not explicitly considered (for example, the

consumption of oxygen by mitochondria). We let the net production rates from these reactions be R_M, R_O, and R_{MO} (moles per unit volume and time). Then the mass balances on M, O, and MO yields equations for the three concentrations N_M, N_O, N_{MO}:

$$\partial N_M/\partial t = \nabla \cdot (D_M \nabla N_M) + R_M - G, \tag{2}$$

$$\partial N_O/\partial t = \nabla \cdot (D_O \nabla N_O) + R_O - G, \tag{3}$$

and

$$\partial N_{MO}/\partial t = \nabla \cdot (D_{MO} \nabla N_{MO}) + R_{MO} + G. \tag{4}$$

In a typical experiment, we might begin in a steady-state situation, governed by Eqs. 2–4 with the time derivatives zero. Then a low temperature is applied to a boundary. This produces a space- and time-dependent temperature T in the tissue, which in turn causes variations in G, the R's, and the D's. To find the effect on the distribution of the N's, we must solve Eqs. 2–4, including the time derivatives, with the given steady state as the initial condition. To do this, we must know the temperature distribution, so we deal with that next.

Temperature Distribution

Consider a semi-infinite slab of tissue occupying the half-space $x > 0$. Initially, the slab has a uniform temperature T_I. At time $t = 0$, the temperature on the boundary $x = 0$ is lowered to T_B and maintained at that temperature. We assume that $T_B < T_F$, where T_F is the freezing point of the tissue. At any time $t > 0$, we will have the following situation: a liquid-solid phase boundary at $x = X(t)$, frozen tissue for $0 < x < X$, and unfrozen tissue for $x > X$. In both the frozen and unfrozen regions there will be a temperature gradient. For simplicity, we assume that the thermal properties of the tissue are piecewise constant, and we use subscript F for the frozen region, and subscript U for the unfrozen region. Then the relevant parameters are the thermal diffusivities D_F, D_U, the specific heats C_F, C_U, and the latent heat L. In general, the solid and liquid densities would also be important, but, again for simplicity, we assume that they are equal. Then the solution for $T(x, t)$ is well known (Carslaw and Jaeger, 1959). It is given most conveniently in terms of a similarity variable

$$\sigma = x/2(D_F t)^{1/2}, \tag{5}$$

and a constant λ to be defined below. The phase boundary is at $\sigma = \lambda$, or $x = X(t) = 2\lambda (D_F t)^{1/2}$, and the temperature is given by

$$T(x,t) = \begin{cases} T_B + (T_F - T_B)\dfrac{erf(\sigma)}{erf(\lambda)} & , \sigma \le \lambda \\ T_I - (T_I - T_F)\dfrac{erfc\,[\sigma(D_F/D_U)^{1/2}]}{erfc\,[\lambda(D_F/D_U)^{1/2}]} & , \sigma \ge \lambda. \end{cases} \tag{6}$$

The constant λ is the unique root of the equation

$$\frac{\exp(-\lambda^2)}{erf(\lambda)} - \frac{C_U D_U^{1/2}(T_I - T_F)}{C_F D_F^{1/2}(T_F - T_B)} \frac{\exp(-D_F\lambda^2/D_U)}{erfc\,[\lambda(D_F/D_U)^{1/2}]} = \frac{L\lambda\pi^{1/2}}{C_F(T_F - T_B)}. \tag{7}$$

For most purposes, it is sufficiently accurate to use constant thermal properties of water and ice for the unfrozen and frozen tissue, respectively. For water, we use the thermal properties at 290 K, about midway between freezing and an initial temperature of $T_I = 310$ K. The values are $D_U = 1.43 \times 10^{-7}$ m^2/s and $C_U = 4{,}180$ J/kg/K. For the frozen tissue, we use the values for ice at $T_F = 273$ K, since most of the events of interest happen at temperatures near the high end of the frozen tissue range. The values are $D_F = 1.15 \times 10^{-6}$ m^2/s and $C_F = 2{,}100$ J/kg/K. The latent heat of fusion is $L = 3.35 \times 10^5$ J/kg. In an experiment using liquid nitrogen for the freezing, we have $T_B = 77$ K. For these values, we find from Eq. 7 that $\lambda = 0.5676$. The temperature profile is then obtained from Eq. 6, and a plot is shown in Fig. 1. The position of the freezing front [$x = 2\lambda(D_F t)^{1/2}$] is about 40 μm for $t = 1$ ms, near 400 μm for $t = 0.1$ s, and just over 1,200 μm for $t = 1$ s.

Kinetics

The kinetic term G in the transport Eqs. 2–4 must be developed in more detail. The reaction is given by Eq. 1, and we assume a second-order forward (combination) reaction, and a first-order reverse (dissociation) reaction. Then

$$G = k_1 N_M N_O - k_2 N_{MO}. \tag{8}$$

The rate constants k_1 and k_2 will in fact vary with temperature. This variation will depend on the particular reaction, but for present purposes it is sufficient to use the van't Hoff law:

$$k_1(T) = k_1(T_I)\exp[-(H_1/R)(1/T - 1/T_I)],$$

and

$$k_2(T) = k_2(T_I)\exp[-(H_2/R)(1/T - 1/T_I)]. \tag{9}$$

Here $R = 8.32$ J/mol/K is the gas constant, and H_1, H_2 are the activation energies for the forward and reverse reactions.

For the general case represented by Eqs. 2–4, one must also develop detailed expressions for R_M, R_O, and R_{MO}. In addition, the temperature dependence of the diffusivities must be dealt with. For the special case of spatially homogeneous reactions considered in this paper, such information is not needed.

For qualitative discussions in the remainder of this paper, we will find it useful to introduce two new temperatures, T_S, and T_E. We define T_S to be the temperature at which the kinetics begins to be affected. This is clearly not a precise concept, but, as a working definition, we take T_S to be the temperature at which the more rapidly varying kinetic coefficient has changed by 10%. Then from Eq. 9 we get

$$T_S = T_I/[1 + (RT_I/H_{max})\log_e(1/0.9)], \tag{10}$$

where H_{max} is the larger of H_1, H_2. The second temperature, T_E, is the temperature at which the cooling process is significantly advanced. Again, this is not precise. For purposes of qualitative discussions, we may define T_E as the temperature at which both kinetic coefficients are no larger than 10% of their original values. Then from Eq. 9 we get

$$T_E = T_I/[1 + (RT_I/H_{min})\log_e(10)], \tag{11}$$

where H_{min} is the smaller of H_1, H_2. These temperatures correspond to values of σ equal to σ_S and σ_E, and to times, for a given x, of

$$t_S = x^2/4D_F\sigma_S^2, \quad t_E = x^2/4D_F\sigma_E^2. \tag{12}$$

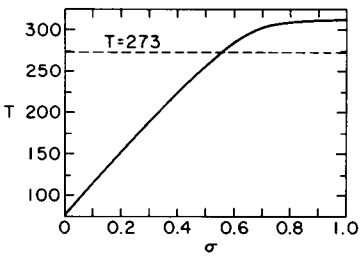

FIGURE 1 Temperature T (Kelvin) vs. the similarity variable $\sigma = x/2(D_F t)^{1/2}$, where x is depth, t the time, and D_F the thermal diffusivity of the frozen tissue. The initial temperature is 310 K and the boundary temperature 77 K. The phase boundary is at $\sigma = 0.5676$.

In subsequent discussions, we will refer to the duration Δt of the cooling event, defined by

$$\Delta t = t_E - t_S = (x^2/4D_F)(1/\sigma_E^2 - 1/\sigma_S^2). \quad (13)$$

III. ANALYSIS FOR HOMOGENEOUS REACTIONS

Reduction of Equations

To simplify the mathematical problem, we assume spatially homogeneous reactions (thus no diffusion terms) and we drop the consumption terms R_M, R_O, and R_{MO}. Then Eqs. 2–4 reduce to ordinary differential equations for the concentrations

$$dN_M/dt = -G, dN_O/dt = -G, \quad dN_{MO}/dt = G. \quad (14)$$

These equations have two immediate integrals: the quantities

$$N_{TM} = N_M + N_{MO} \quad (15)$$

and

$$N_{TO} = N_O + N_{MO} \quad (16)$$

are both constant in time. These two integrals allow the reduction of Eq. 14 to a single first-order equation, which is best written in terms of the fraction saturation

$$S = N_{MO}/N_{TM}. \quad (17)$$

The equation is

$$dS/dt = k_1 N_{TM}(S_M - S)(1 - S) - k_2 S, \quad (18)$$

where

$$S_M = N_{TO}/N_{TM}. \quad (19)$$

Because S_M is the ratio of total O to total M, it is an upper bound on the saturation for given N_{TO} and N_{TM}.

The basic mathematical problem is to solve Eq. 18, where S_M and N_{TM} are given, and k_1, k_2 are determined by Eq. 6 for $T(x, t)$ and Eq. 9 for $k_1(T)$, $k_2(T)$. In addition, we must specify an intial condition for S: $S(0) = S_I$. We suppose further that, before the cooling starts, the system is in steady state (which in this case means equilibrium). Then S_M and S_I satisfy a relation obtained by setting the right-hand side of Eq. 18 to zero at $t = 0$:

$$S_M = S_I + k_2 S_I/[k_1 N_{TM}(1 - S_I)]. \quad (20)$$

Conditions for Successful Capture

Consider now the conditions necessary for small changes in saturation during freezing. To begin with, there is the obvious condition that the boundary temperature T_B be sufficiently low to stop the reaction. Thus both of the kinetic times $[N_{TM}k_1(T_B)]^{-1}$ and $[k_2(T_B)]^{-1}$ must greatly exceed the typical storage time of the specimen (assuming that storage is also at temperature T_B). This condition is necessary but not sufficient, since changes in saturation will occur during the cooling. If the duration of the cooling is much less than the kinetic time scales, the change in S will be very small. If, on the other hand, cooling is slow compared with the kinetics, then it is possible to get significant changes in saturation. The duration of the cooling was discussed in section II, Kinetics, and the time is given by Eq. 13. The most important thing to note about Δt is its rapid increase with depth x. This is a direct consequence of the diffusive spread of the temperature profile as it propagates into the tissue. The duration Δt should be compared with the kinetic times. More specifically, we can assert that very little change in S will take place during cooling provided Δt is much less than the shortest kinetic time scale. The shortest kinetic time scales occur at the high initial temperature. The reciprocals of these scales are $k_1(T_I)N_{TM}$ and $k_2(T_I)$. For accurate capture of the reaction, we require that $(\Delta t)^{-1}$ be much greater than the larger of these. A sufficient conditon, symmetric in the two rates, is

$$1/\Delta t \gg K = k_1(T_I)N_{TM} + k_2(T_I), \quad (21)$$

or

$$x^2 \ll (4D_F/K)/(1/\sigma_E^2 - 1/\sigma_S^2). \quad (22)$$

Eq. 22 defines the spatial region in which capture of the state by cooling is assured. The completion of the discussion requires values of σ_S and σ_E. As we shall see in an example in section III, for the particular case of oxygen and myoglobin with liquid nitrogen providing the cooling, we get $T_S = 300$ K and $T_E = 247$ K, so that $\sigma_S \approx 0.7$, $\sigma_E \approx 0.5$. For these values, Eq. 22 may be approximated by

$$x^2 \ll 2D_F/K. \quad (23)$$

When the condition (Eq. 23) is satisfied, cooling should capture the state with very little change in saturation.

In general, the violation of the inequality (Eq. 23) will be associated with significant changes in saturation. There is one exceptional case, however, that deserves special notice. If $H_1 < H_2$, then the saturation will increase during freezing. If, in addition, the initial value S_I and maximum value S_M are close, the reaction is constrained by the depletion of the reactant O. Then the maximum possible change in S is small, and the inequality (Eq. 23) becomes irrelevant. The condition for this situation is $S_M - S_I << S_I$, or, from Eq. 20,

$$k_2(T_I) \ll N_{TM}k_1(T_I). \quad (24)$$

Thus a successful capture can occur even with slow cooling (Eq. 23 violated), provided (*a*) equilibrium saturation shifts upward with decreasing temperature ($H_1 < H_2$), and (*b*) the dissociation reaction is much slower than the combination reaction ($k_2 << N_{TM}k_1$).

An Example: Oxygen and Myoglobin

By way of example, we apply the concepts developed so far to the reaction of oxygen and myoglobin in skeletal muscle, with the reaction being captured by freezing with liquid nitrogen. The thermal parameters are then the same as in section II, Temperature Distribution. For the kinetic parameters, we use the following values: rates $k_1 = 2.40 \times 10^{10}$ cm^3/mol/s and $k_2 = 65$ s^{-1} at $T = 310$ K, and activation energies $H_1 = 5.5$ kcal/mol, $H_2 = 19$ kcal/mol (Antonini, 1965). For the myoglobin concentration N_{TM}, we use the three values 5×10^{-7}, 5×10^{-8}, and 5×10^{-9} mol/cm^3, which cover the range of interest for skeletal muscle (Wittenberg, 1970).

Consider first the reaction rates at the final temperature. From Eq. 9 we get $[k_2(T_B)]^{-1} = 1.5 \times 10^{31}$ yr, $[N_{TM}k_1(T_B)]^{-1} = 502$ d for $N_{TM} = 5 \times 10^{-7}$, and longer for the other values of N_{TM}. Even though the applicability of Eq. 9 over such a wide temperature range is somewhat questionable, the margin is large enough to be confident that $T_B = 77$ K is low enough to stop the reaction.

We consider each of the three values of myoglobin concentration in turn. For $N_{TM} = 5 \times 10^{-9}$ mol/cm^3 (a very low value for skeletal muscle), the inequality (Eq. 23) becomes $x << 112$ μm. To verify this condition for capture, we have used the Runge-Kutta method to integrate Eq. 18 numerically, for an initial value $S_I = 0.5$. Fig. 2 (curve *a*), shows S_F, the final saturation after complete cooling, as a function of depth. The change induced by freezing is <1% for x up to 25 μm, <3% for x up to 50 μm, and a little over 10% at $x = 100$ μm.

For a larger myoglobin concentration, $N_{TM} = 5 \times 10^{-8}$ mol/cm^3, the condition for capture (the inequality Eq. 23) is $x << 43$ μm. Again we

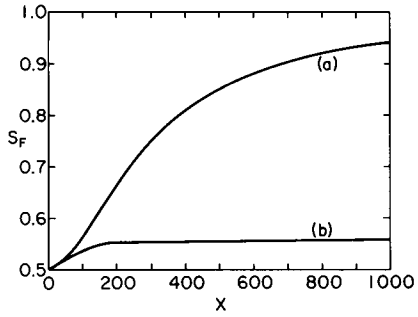

FIGURE 2 Final myoglobin saturation S_F vs. depth x (μm) for initial saturation 0.5. Myoglobin concentration of 5×10^{-9} mol/cm^3 (*a*), and 5×10^{-8} mol/cm^3 (*b*). The initial temperature is 310 K and the boundary temperature 77 K.

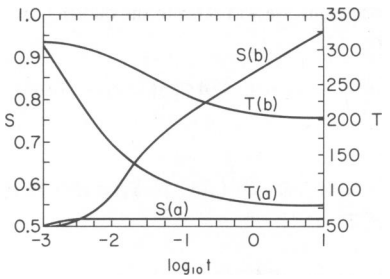

FIGURE 3 Myoglobin saturation S and temperature T (Kelvin) vs. time t (in seconds) at a depth of 50 μm for a boundary temperature of 77 K (*a*) and 197 K (*b*). Myoglobin concentration of 5×10^{-9} mol/cm^3.

have verified this by a numerical integration, and Fig. 2 (curve *b*) shows the final saturation S_F for an initial saturation $S_I = 0.5$. For those values, the maximum saturation (saturation at oxygen depletion) is $S_M = 0.5542$. For $x > 250$ μm, the saturation reaches this value. For a freezing change of <1%, the numerical calculations show that we must have $x \leq 25$ μm, and the change is around 10% at $x = 80$ μm.

For $N_{TM} = 5 \times 10^{-7}$ mol/cm^3, a value of myoglobin concentration typical of red skeletal muscle, we have $k_2/(k_1 N_{TM}) = 5.4 \times 10^{-3}$, hence $S_M - S_I << S_I$, and the situation is entirely constrained by available free oxygen. For $S_I = 0.5$, for example, we get (from Eq. 20) a maximum saturation of $S_M = 0.5054$. Because $H_1 < H_2$, the saturation will increase during freezing, and it is therefore constrained to be between S_I and S_M, a variation of only ~1%. Thus the parameter values for this case are particularly fortunate for the capture of reactions by freezing.

As an example of the effect of boundary temperature, we also consider the case $T_B = 197$ K, corresponding to dry ice. Then the kinetic times at the final temperature are $[N_{TM} k_1(T_B)]^{-1} = 0.014$ s (for $N_{TM} = 5 \times 10^{-7}$ mol/cm^3), and $[k_2(T_B)]^{-1} = 8.4$ days. Clearly the dry-ice temperature is not low enough to capture this reaction. It is true that, for this value of N_{TM}, the oxygen-depletion constraint discussed above will limit the change of S during the experiment. However, subsequent storage at dry-ice temperature would allow the still-active specimen to pick up environmental oxygen. For lower values of N_{TM}, the situation is worse. Fig. 3 (obtained by numerical integration of Eq. 18) compares, for $N_{TM} = 5 \times 10^{-9}$, the liquid nitrogen case (curves marked *a*) with the dry-ice case (curves marked *b*). Both the saturation S and temperature T are shown as functions of time, at a depth of $x = 50$ μm. In the dry-ice case, the saturation continues to increase rapidly even after the temperature has dropped to within a few degrees of T_B. The case for using a low boundary temperature is clear!

IV. DISCUSSION

Concentration Gradients

In the present calculations, concentration gradients have been ignored. There are actually two possible sources of such gradients. First, the existing steady state that is to be captured by freezing may have concentration gradients. This important case will be dealt with in a future paper. Second, even in an initially homogeneous case, concentration gradients will be produced by the cooling process itself. Just recall that the saturation changes discussed in an example in section III are depth dependent. If these gradients were large, then the assumption of spatially homogeneous reactions would not be consistent. We show, with some simple estimates, that the cooling-induced concentration gradients are in fact small enough to be safely ignored. The basic idea is straightforward. Mass diffusion is much slower than thermal diffusion, so there is little time for mass rearrangement during the cooling process.

Consider the estimates that establish this. As discussed in section II, Kinetics, we may identify a temperature T_S, somewhat below the initial temperature, at which the kinetic rates have begun to change, and a lower temperature T_E at which significant changes in the kinetic rates have taken place. These temperatures will correspond to fixed values σ_E and σ_S of the similarity variable σ (Eq. 5). At time t, these two temperatures will be located at $x_E = 2\sigma_E(D_F t)^{1/2}$ and $x_S = 2\sigma_S(D_F t)^{1/2}$. The thickness of this region of active change will be

$$\Delta x = x_S - x_E = 2(\sigma_S - \sigma_E)\,(D_F t)^{1/2}, \tag{25}$$

and its center will be roughly at

$$x_C = 0.5(x_E + x_S) = (\sigma_E + \sigma_S)\,(D_F t)^{1/2}. \tag{26}$$

The duration of the event at x_C is given by Δt (Eq. 13) with $x = x_C$, and this is the time available for mass diffusion in the vicinity of x_C. If D is the maximum mass diffusion coefficient of the three components M, O, and MO, then the characteristic time t_D required for mass diffusion over a distance Δx is, in order of magnitude

$$t_D = (\Delta x)^2/D = 4(\sigma_S - \sigma_E)^2 D_F t/D. \tag{27}$$

Thus the ratio of time available to time required for mass diffusion is

$$\Delta t/t_D = \frac{D}{D_F}\,\frac{(\sigma_E + \sigma_S)^3}{16(\sigma_S - \sigma_E)\,(\sigma_E \sigma_S)^2}. \tag{28}$$

The numerical coefficient involving the σ's is of order 1. [For example, for the values $\sigma_S = 0.7$ and $\sigma_E = 0.5$ chosen in section III, in Conditions for Successful Capture, Eq. 28 becomes $\Delta t/t_D = 4.4\,(D/D_F)$.] Because the mass diffusion coefficient D is generally much less than the thermal diffusivity D_F, there will be negligible mass diffusion during the cooling process. For example, for oxygen and myoglobin, the maximum D is that of free oxygen, $D = 2 \times 10^{-5}$ cm^2/s—so that $D/D_F = 1.7 \times 10^{-3}$.

Other Aspects of the Thermal Problem

In the thermal calculations of section II, Temperature, it was assumed that cooling proceeds at a fixed low boundary temperature. This requires good thermal contact between the heat sink and the specimen. Two factors that could degrade the thermal contact are a frost layer, or a vapor or air layer. Consider first the frost layer. The thermal properties of the frost are close to those of the frozen tissue. Thus in the theory of section III, we may correct for the frost layer by interpreting the depth x to be the sample depth plus the thickness of the frost layer.

Correcting for a vapor or air layer is more difficult and we do not consider it in detail here. In any case it is something to be avoided if possible, since it could lead to appreciable slowing of the cooling process, which in turn decreases the size of the region in which the initial state is correctly captured.

All of the calculations here have assumed the tissue to be a semi-infinite slab cooled at one boundary. A more realistic configuration is that of a slab of finite thickness either (*a*) cooled at both boundaries, or (*b*) cooled at one boundary and insulated at the other. These two cases are in fact the same. By symmetry, case (*a*) with width $2W$ is the same as case (*b*) with width W. The problem is made much more difficult by finite slab width. An approximate treatment is discussed briefly by Carslaw and Jaeger (1959, p. 293). A full numerical treatment would be feasible but laborious. Wollenberger et al. (1960) treated the case of finite slab width, but they neglected the latent heat of fusion, an approximation that leads to serious errors in the temperature distribution.

For short times, the solution for a semi-infinite slab can be used to describe a finite slab. From Fig. 1, we see that, for our particular example, the temperature is still approximately equal to the initial temperature for $\sigma \geq 1$. Thus the effects of the boundary cooling only penetrate to $\sigma = 1$, that is, to $x = 2(D_F t)^{1/2}$. Now consider a slab of width W insulated on one boundary (or a slab of width $2W$ cooled from both boundaries). Then the condition for using the present theory is that σ at W be one or greater: $W \geq 2(D_F t)^{1/2}$, or

$$t \leq W^2/4D_F. \tag{29}$$

We may translate this into a condition on sample depth. Once again we use the temperature T_E at which appreciable changes in the kinetics have taken place. This temperature, corresponding to $\sigma = \sigma_E$, occurs at depth x at time $t = x^2/(4\sigma_E^2 D_F)$. This will be in the range of validity of the theory provided that $x^2/(4\sigma_E^2 D_F) \leq W^2/4D_F$, or

$$x \leq \sigma_E W. \tag{30}$$

If, for example, $T_E = 250$ K, then $\sigma_E = 0.5$, and samples to a depth of about $0.5W$ are reliably analyzed with the theory presented here for a semiinfinite slab. It is important to note that these numbers may be somewhat different for different boundary and initial temperatures, but the same concepts will allow the calculation for other cases.

Myoglobin as an Oxygen Indicator

We consider briefly the implications of the present calculations for the use of myoglobin saturation as an indicator of oxygen concentration. As the results of section III show, the myoglobin concentration is a crucial parameter. For very low myoglobin concentrations (5×10^{-9} mol/cm^3), typical of fibers of very low aerobic capacity, accurate capture of the state by cooling at liquid nitrogen temperature requires a shallow sample depth (on the order of 50 μm or less; see Fig. 2 *a*). If dry ice is used instead of liquid nitrogen, the situation is much worse. There are then appreciable errors induced by cooling even at a depth of 50 μm (see Fig. 3 *b*). Actually, such low myoglobin concentrations are difficult for another reason: they give very weak signals for the spectrophotometric measurements.

The situation for red skeletal muscle of large animals such as dog and man is very much better, since the myoglobin concentration is around 5×10^{-7} mol/cm^3. Then, as the analysis in section III shows, the limiting factor is the depletion of free oxygen. Even if all the initially free oxygen becomes bound to myoglobin during cooling, an initial saturation of 0.5 increases only to about 0.505, well within the limits of experimental error. In this oxygen-constrained regime, parameters such as sample depth and boundary temperature become unimportant. There is simply no possibility of a significant shift in saturation during cooling.

The above conclusions are consistent with the experimental results of Gayeski and Honig (1981). They measured myoglobin saturation in dog gracilis muscle, a tissue with a myoglobin concentration on the order of 5×10^{-7} mol/cm^3. They found that the distribution of saturation values measured in a given sample was the same at a depth of 50 μm as at 500 μm. They also compared saturation measurements in two gracilis muscles from the same dog, when one was frozen with a copper block at liquid nitrogen temperature and the other with the block at dry ice temperature. Again they found identical distributions of myoglobin saturation values.

One major difference between the present theoretical work and the experiments is the presence of concentration gradients in the experiments. Until the theory is extended to include such gradients, we cannot draw any final conclusions about the capture of concentration gradients by cooling. However, the mechanism discussed above, namely, the limiting of saturation changes by oxygen depletion, is operative in more complicated situations with gradients. Thus there is reason to be optimistic that myoglobin saturation is a good oxygen indicator, even when concentration gradients are present, provided the myoglobin concentration is high.

Summary

The question studied here is fundamental in the understanding of the cryophotometric technique for capturing the state of living tissue. The question is the following: What are the conditions necessary to ensure small changes in the chemical state of tissue during freezing? The model used here is a semi-infinite slab of tissue in which there is a spatially homogeneous chemical reaction. The tissue is frozen by the application of a low temperature to the boundary. The kinetic equations for a reaction of the form $M + O \rightleftarrows MO$ are analyzed for a freezing process, with temperature-dependent rate constants, and a temperature distribution determined from the heat equation. The calculations show that regions near the boundary are cooled rapidly and experience little change in composition during cooling. Regions further from the boundary may undergo significant changes in composition during the freezing. The length scale that separates near and far in this context is, in order of magnitude, given by $(2D_F t_K)^{1/2}$, where D_F is the thermal diffusivity of the frozen tissue, and t_K is the shortest time scale associated with the chemical kinetics. More precisely, if x is the depth into the tissue from the cooled boundary, then there will be little change of saturation during freezing for $x << (2D_F t_K)^{1/2}$, and there may be a significant change for $x > (2D_F t_K)^{1/2}$.

Other factors can influence the change in composition during freezing. If the reaction is such that saturation tends to increase with decreasing temperature, then the change during freezing can be limited by the depletion of one of the reactants. This happens, for example, in muscle tissue with a high myoglobin concentration, where the change in saturation during freezing is limited by the depletion of oxygen.

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