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A Mathematical Model of in situ Freezing in Liquid Nitrogen

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ABSTRACT

In situ freezing is a procedure, typically applied in neuroscience, to halt metabolism and diffusion. However, the freezing process is not instantaneous, and the regional concentrations of a compound under study may change before the tissue is completely frozen. Knowing the local freezing time, metabolic rate and the diffusion coefficient of the compound of interest, it should be possible to reconstruct the spatial concentration profile prevailing before the object was placed in the cryogen.

A mathematical model for calculating the temperature changes at different depths in rabbit and rat heads cooled in liquid nitrogen has been developed. By comparing with experimental results it has been found that the mathematical model can be used for prediction of the local freezing time with a small error.

INTRODUCTION

In situ freezing is a standard procedure for halting diffusion and metabolism (7, 14, 15, 17, 18).

Since freezing is a rather slow process, the regional concentration of a given substance measured *post mortem* may differ significantly from the true *in vivo* levels (5, 6, 18). In order to retrospectively estimate the levels of biologically labile and/or diffusible compounds at the time the object was put into the cryogen, it is necessary to know at what time a certain temperature was reached.

The process of cooling an object with a cryogenic fluid can be divided into two parts: heat transfer from the surface of the object to the cryogen by convection and heat transfer by conduction through the object to its surface. In small objects it is usually the transfer of heat from the object to the cryogen that limits the cooling – this is often referred to as convection-limited cooling. The cooling of large biological objects, although initially convection-limited, is limited by the rate of conduction through the object to its surface – often referred to as conduction-limited cooling (1).

In a previous paper results from temperature tracings at different depths in phantoms (apples) submerged in different well-known cryogenic liquids were reported (11). Further, in order to predict the temperature-time relationship at different distances from the surface of rabbit and rat heads submerged in liquid nitrogen (which was reported to give the shortest freezing times), a mathematical model was developed and applied. We showed results from rat and rabbit heads submerged in liquid nitrogen, and that the calculated values were close to the measured ones. The model should be typically useful when considering autoradiographic assessments of blood flow (5, 6, 9, 12). In this paper I report the

details of the theory.

THEORY

Below we consider the problem of heat conduction in a homogeneous material containing two phases (liquid and solid), corresponding to unfrozen and frozen tissue.

Let $T=T(x, t)$ be the temperature at each point x, with the coordinates (x_1, x_2, x_3), in a given object at time, t. If ρ is the density (kg/m^3) and c the specific heat capacity (J/kg/K), in a system where there is no convection, then the change in thermal energy (J/m^3) per unit time (s) can be expressed as

$$\frac{\partial}{\partial t}(\rho c T) = div\left(\lambda\, grad\, T\right) + p \qquad [I]$$

where λ is the heat conductivity (W/m/K) and $p=p(x, t)$ is the heat production (W/m^3) at the point x (m) at time t. Setting ρ, c and λ as constants, we get

$$\frac{\partial T}{\partial t} = \Delta a T + \frac{p}{\rho c} \qquad [II]$$

where

$$\Delta = \frac{\partial^2}{\partial x_1{}^2} + \frac{\partial^2}{\partial x_2{}^2} + \frac{\partial^2}{\partial x_3{}^2} \quad \text{and} \quad a = \frac{\lambda}{\rho c} \qquad [III]$$

Eq. [II] can be written as

$$\frac{\partial T}{\partial t} = a\left(\frac{\partial^2 T}{\partial r^2} + k\,\frac{\partial T}{r\,\partial r}\right) + \frac{p}{\rho c} \qquad \text{[IV]}$$

where k=1 for an infinite cylinder and 2 for a sphere.

The heat diffusivity (a) of biological objects depends on parameters such as water content and temperature [2, 13]. The values for unfrozen (liquid) and frozen (solid) tissue used in this work were the same as the reported values for mashed potatoes (2) (See Table 1.).

Boundary and initial conditions

$T(r_m, t)$, where T is the temperature (°C), r_m is the radius of the object (m) and t is the time (s), was set to be equal to the following empirically derived formula (from temperature measurements with a Type K thermocouple connected to a Fluke 51 (Fluke) on the surface of apples):

$$T(r_m, t) = T(r_m, \infty) + 0.7\cdot(T(r, 0) - T(r_m, \infty)) - 0.3\cdot(T(r, 0) - T(r_m, \infty))\cdot((t-8)/8)^3,$$

with the constraint that [V]

$T(r_m, t) \geq T(r_m, \infty)$.

$T(r_m, \infty)$ was set to -195 °C and $T(r, 0)$ was set equal 21 °C (for apples), 33 °C (for rats) or to 39 °C (for rabbits), these being the approximate measured temperatures of the objects immediately prior to immersion.

Phase transition

At a slow rate of cooling, it has been reported that the ice formation of most foodstuffs is initiated between -1 and -3 °C and then continues over a certain range of temperature (2). In very fast freezing (freezing in liquid nitrogen), the rate of ice formation is very probably not only dependent on the rate at which heat is transported away but is also dependent on the rate of movement of the water molecules forming a "primary ice" and the rate at which this primary, non-stable, ice structure forms a more stable structure – such a system would be expected to be highly non-linear.

Thus, to simulate the process physically at a microscopic level is probably very difficult. Therefore, it was decided to use a model as simple as possible – assuming there is no freezing zone and that the change in enthalpy occurs when the "primary ice" forms a more stable structure. The liberation of heat was taken to be dependent, in an exponential manner, on the total time that the local temperature has been below the freezing point, *fp*. Further, the rate of heat transport can be larger than the rate of heat formation and the rate of heat liberation can exceed the rate at which the heat is transported away, but because of the constraints, the liberation cannot bring the local temperature back above *fp*. In the present case *fp* was assumed to be -2 °C.

The value of the latent heat of fusion *(H)* in biological objects seems to be proportional to the content of freezable water (2). That is, if the content of freezable water is 50%, the latent heat will be 50% of the amount of heat liberated when pure water freezes. In my calculations, the value of H was set to 268 J/g (assuming that the content of freezable water is 80%) (2).

Expressing the above in terms of formulae:

$$\frac{dH}{dt} = p \qquad \text{[VI]}$$

where $p = 0$ if $T(r, t) > fp$, otherwise

$$p = Q e^{-kt^*} \qquad \text{[VII]}$$

where k and Q are constant and t^* is the total time the local temperature has stayed below fp. Q was set equal to 61.64 W/g resulting in a value of k equal to 0.23 s^{-1}. The values of these two constants were chosen by interactively fitting calculated temperature-time profiles to measured ones for apples (containing about the same amount of water as rabbit and rat heads) (11). However, changing these constants, while keeping H constant, by a factor of 25 led to a minimal difference in the freezing times. There may be a physically more accurate model for heat liberation, but finding it was beyond the scope of this work.

Solution

The system was solved numerically on a Power Macintosh 8500/120 by using an explicit finite difference procedure (spherical coordinates for apples and cylindrical coordinates for rabbit and rat heads). We estimated the average radius of the objects by measuring with a ruler. The radius of the cylinder was set to 20 mm when simulating the cooling of a rabbit head for the case where the skin had been left intact and 18 mm for the case where the skin had been removed. For the rat head (without skin) the radius was set to 11 mm. For experimental details see

(11). The values of the parameters used in the calculations are listed in Table 1.

RESULTS

Both calculated and measured temperature-time courses are shown in Figs 1, 2 and 3. It can be deduced that the experimental and theoretical values compare well.

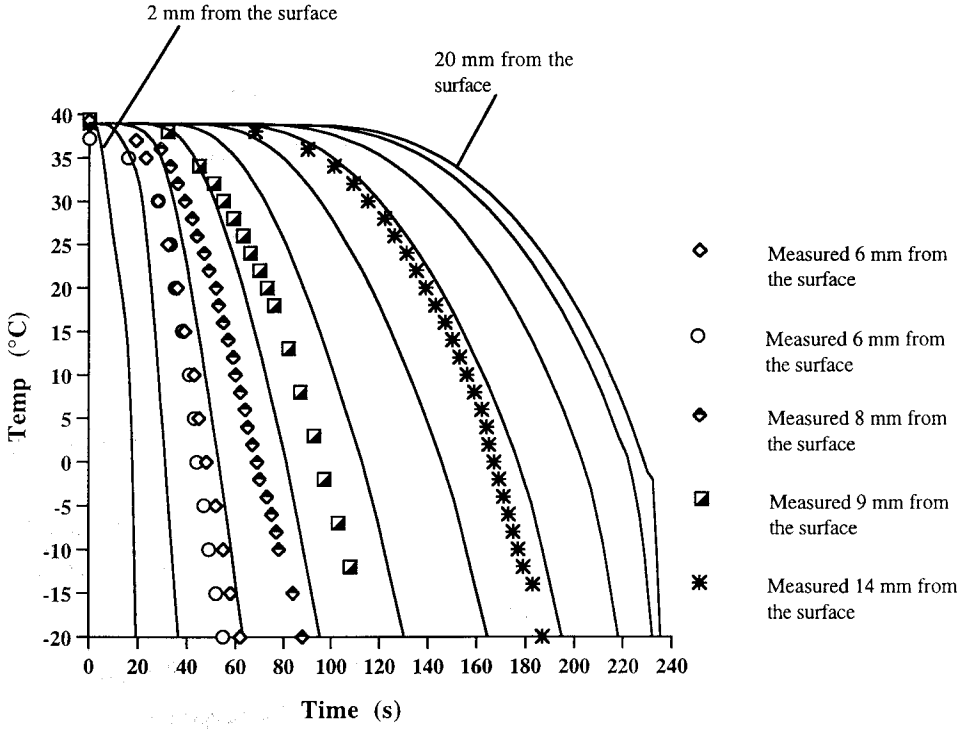

Fig. 1. Calculated and measured temperature-time courses at different depths in intact rabbit heads. Calculated courses: The distance between neighbouring courses is 2 mm. "20 mm from the surface" is equal to the very center.

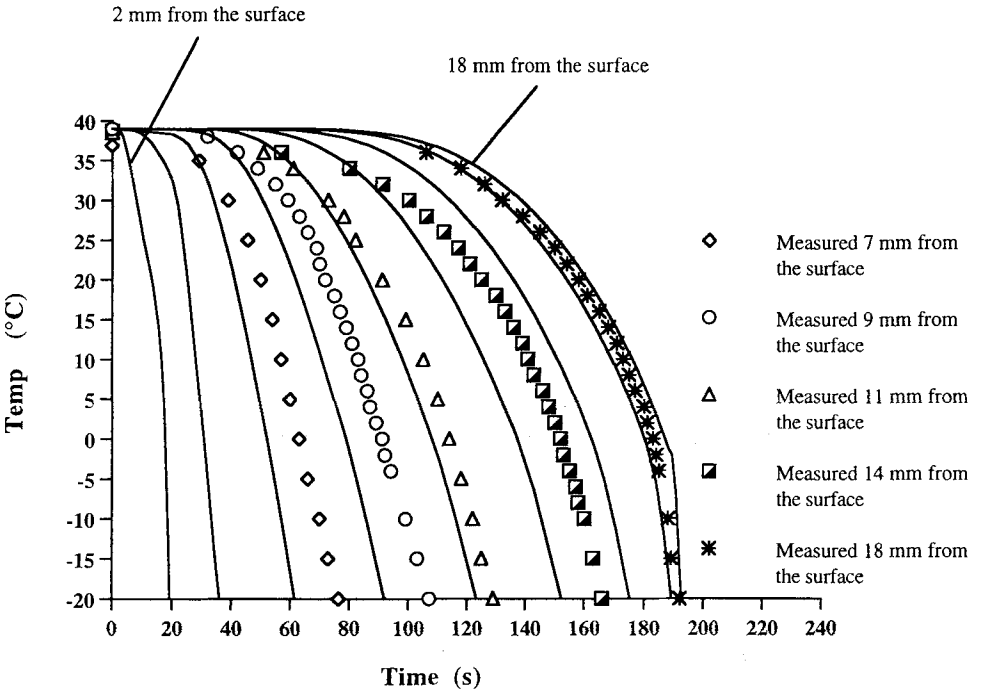

Fig. 2. Calculated and measured temperature-time courses at different depths in stripped rabbit heads. Calculated courses: The distance between neighbouring courses is 2 mm. "18 mm from the surface" is equal to the very center.

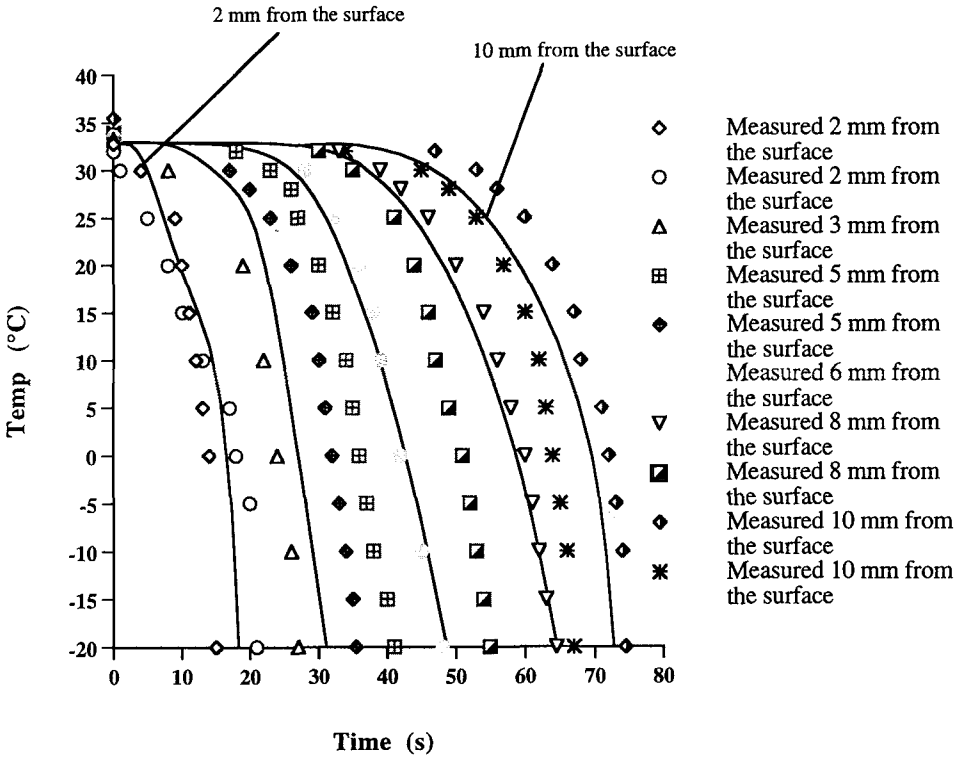

Fig. 3. Calculated and measured temperature-time courses at different depths in stripped rat heads. Calculated courses: The distance between neighbouring courses is 2 mm. "10 mm from the surface" is equal to the very center.

Table 1. Values of the parameters used in the calculations.

Parameter	Value	Unit	Physical quantity
a_{liquid}	1.39×10^{-7}	m^2/s	thermal diffusivity*
a_{solid}	1.042×10^{-6}	m^2/s	thermal diffusivity*
c_{liquid}	3.6×10^3	J/kg/K	specific heat capacity*
c_{solid}	1.92×10^3	J/kg/K	specific heat capacity*
fp	-2.00	°C	temperature*
H	268×10^3	J/kg	latent heat of fusion*
λ_{liquid}	0.5	W/m/K	thermal conductivity*
λ_{solid}	2.0	W/m/K	thermal conductivity*
ρ_{liquid}	1.00	kg/dm^3	density*
ρ_{solid}	1.00	kg/dm^3	density*
$T(r_m, \infty)$	-195	°C	border temperature
$T(r, 0)$	21.0, 33.0 or 39.0	°C	initial temperature

* denotes the values which have been taken or derived from studies on mashed potatoes (2). Since mashed potatoes have a water content similar to that of apples and the brain, these values should be appropriate.

DISCUSSION

By knowing the time over which a substance has been allowed to diffuse and/or be metabolized, it should be possible, at least roughly, to reconstruct the spatial concentration profile before the object was placed in the cryogen. However, the change in temperature with time in a biological object, suddenly exposed to an environment at a very low temperature, is dependent on several factors (not sufficiently understood) in a complex manner. Nevertheless, although the heads are not perfect cylinders, the applied mathematical model seems to be useful for predicting the freezing time at different depths.

Well-known models of the type shown by Comini et al (2) were found to generate too long freezing-times (data not shown). This is because of the fact

that in such a model the heat has to be transported away via a freezing-zone (probably very narrow in rapid freezing), with a lower value of the heat diffusivity than completely frozen tissue. In the model developed by the author of this paper, the local region obtains the heat diffusivity of ice instantaneously, although the heat still is to be liberated.

The model shown in this paper is useful when correction for differences in freezing time is necessary, e.g. autoradiographic assessment of cerebral blood flow (9, 12, 16).

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