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Theoretical local freezing times of small rodent brains submerged *in situ* in liquid nitrogen[1]

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ABSTRACT

In situ freezing is a standard procedure, typically applied in neuroscience, to stop post-mortem metabolism and diffusion. However, the concentration of a compound under study may well change before the tissue is completely frozen. Knowing the approximate local freezing time should make it possible to control this problem. A mathematical model of *in situ* freezing in liquid nitrogen has recently been introduced, and freezing times derived from this model are presented here. The hope is that this information will be considered useful when *in situ* freezing of small rodent brains is applied.

INTRODUCTION

In situ freezing is a standard procedure used to terminate metabolism and diffusion (6, 7, 8). Since the freezing procedure is a relatively slow process, even in liquid nitrogen, the regional concentration of a given substance may have time to change considerably before a sufficiently low temperature has been reached (2, 9). Recently, a mathematical model useful for predicting the local freezing time of objects sub-merged in liquid nitrogen has been introduced (5). This model has been implemented in a study on blood flow in small ischemic regions in the rabbit brain (4). However, a more general applicability of the model is suppressed by the fact that the mathematics involved are somewhat awkward and must be solved numerically. Therefore, in order to make it easier to estimate local freezing-times, computed local freezing-times are published.

THEORY AND SOLUTION

For details about theory, see Roos (5). In the present work, the initial object temperature was set to 33, 37, or 41°C. Otherwise the parameter values were the same as previously (5). The calculations were performed using an explicit finite difference procedure.

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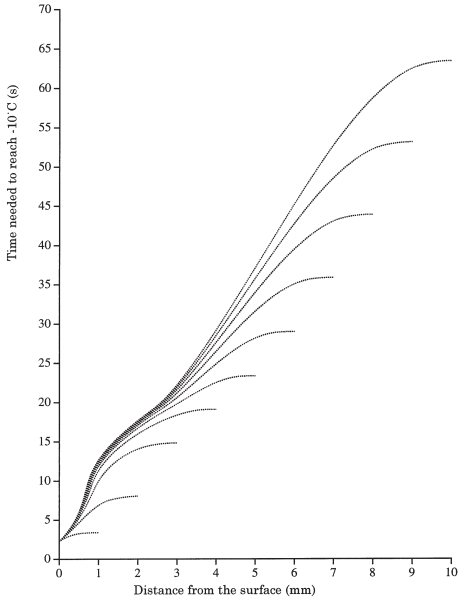

Fig. 1A. Objects with radii from 1 (lowest)–10 (highest) mm. Time required to reach –10°C (freezing time) at different depths from the surface of cylindrical objects, containing 80% water, submerged in liquid nitrogen. Initial object temperature was 33°C.

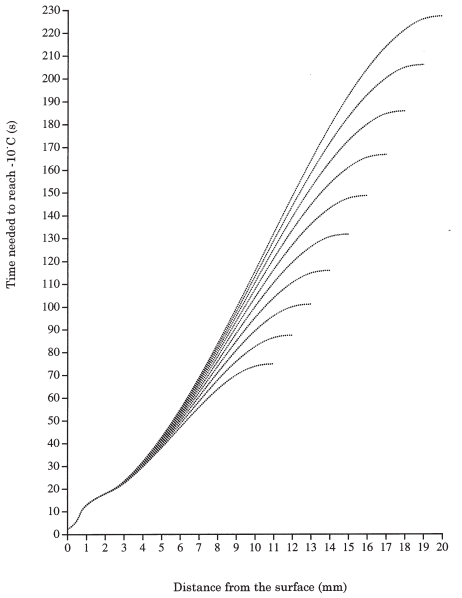

Fig. 1B. Objects with radii from 11 (lowest)–20 (highest) mm. Time required to reach –10°C (freezing time) at different depths from the surface of cylindrical objects, containing 80% water, submerged in liquid nitrogen. Initial object temperature was 33°C.

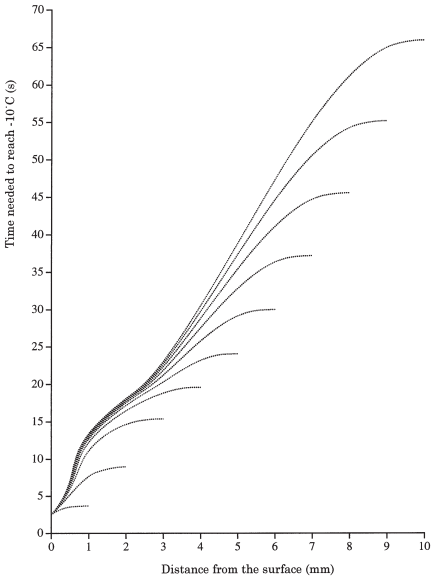

Fig. 2A. Objects with radii from 1 (lowest)–10 (highest) mm. Time required to reach –10°C (freezing time) at different depths from the surface of cylindrical objects, containing 80% water, submerged in liquid nitrogen. Initial object temperature was 37°C.

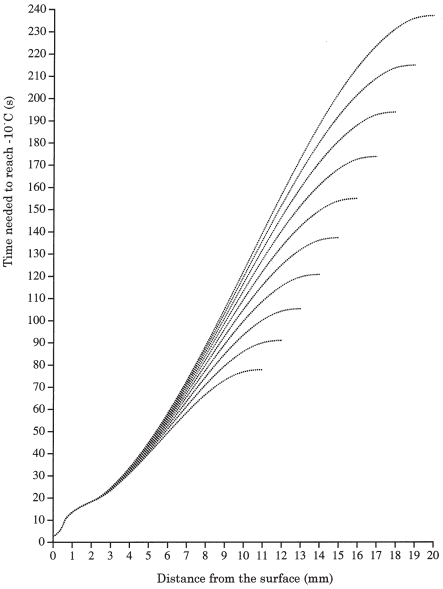

Fig. 2B. Objects with radii from 11 (lowest)–20 (highest) mm. Time required to reach –10°C (freezing time) at different depths from the surface of cylindrical objects, containing 80% water, submerged in liquid nitrogen. Initial object temperature was 37°C.

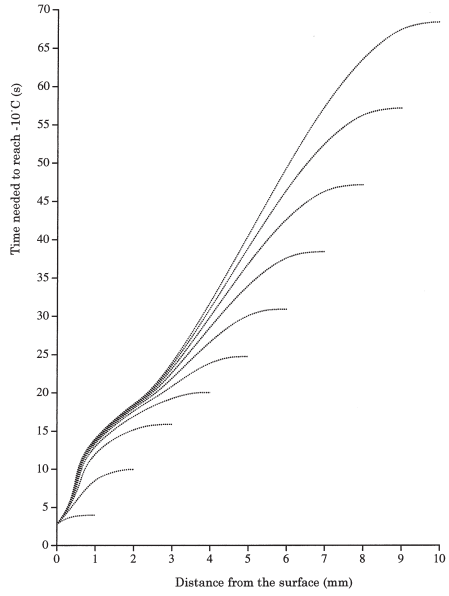

Fig 3A. Objects with radii from 1 (lowest)–10 (highest) mm. Time required to reach –10°C (freezing time) at different depths from the surface of cylindrical objects, containing 80% water, submerged in liquid nitrogen. Initial object temperature was 41°C.

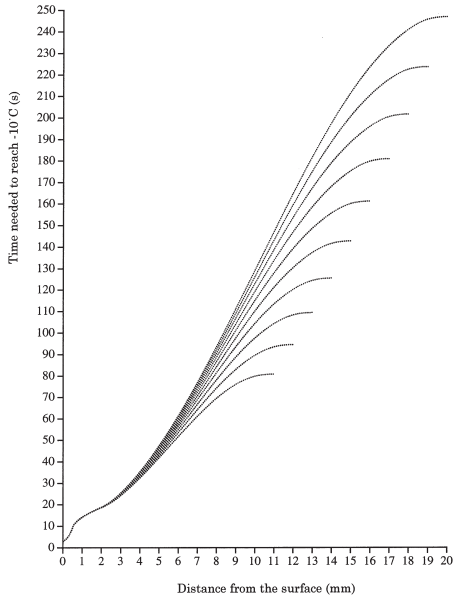

Fig 3B. Objects with radii from 11 (lowest)–20 (highest) mm. Time required to reach –10°C (freezing time) at different depths from the surface of cylindrical objects, containing 80% water, submerged in liquid nitrogen. Initial object temperature was 41°C.

RESULTS AND DISCUSSION

Computed local freezing times, the time required to reach –10°C, for cylindrical objects with different initial temperatures are shown in Figs. 1A–3B. While neither the rabbit head nor rat head is a perfect cylinder, a cylindrical approximation seems to generate freezing times that fit well with those measured previously (3). However, as noted earlier, fur may act as an insulator (although not in the case of the rabbit), thus making freezing times less predictable. Consequently, in order to have control over the freezing times, fur should be removed prior to freezing, except with the rabbit, where it has been shown that fur does not cause any problem (3).

Knowing the local freezing time means that errors due to post-mortem diffusion and/or metabolism can be estimated. The information shown in this paper is typically useful when considering procedures such as auto-radiography of cerebral blood flow using the iodoantypyrine method (4).

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