A NEW MODEL FOR STUDYING THE PRIMARY EFFECTS OF COOLING STRESS ON THE ABILITY OF ORGANISMS TO ENTER INTO CRYOBIOISIS AND ULTIMATELY ANHYDROBIOISIS

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ABSTRACT

The ability of organisms to survive through severe environmental extremes is now of great interest, leading to new advancements for drug delivery systems, cryogenics, and food stabilization. However, despite minor headways into elucidating the events involved in severe stress survival, the specific processes remain unclear.

The present investigation involved utilizing the nematode Panagrellus redivivus as a new model for cryo-and anhydrobiotic survival. Following cold-acclimation of nematodes from 23°C to either 15°C or 5°C, through the use of 1°C, 2°C, and 3°C daily incremental reductions in temperature, various dehydration regimes were implemented. Nematodes cooled at 2°C/day and nematodes acclimated to 5°C rather than 15°C were better able to survive dehydration. In addition, with respect to the initial, 86%, 72%, 62%, 55%, 0% relative humidity (r.h.) regime, successively faster dehydration regimes did not support survival. Present results indicate that a regime involving higher initial humidities (i.e. 97%, 93%, 86% r.h., etc.) for longer durations prior to exposure to reduced r.h. levels may be an applicable direction for future research.

INTRODUCTION

In 1959 there was a serious attempt to compile studies on both prokaryotic and eukaryotic organisms able to survive in extremely harsh conditions (Keilin, 1959). The organisms concerned contend with their severe surroundings by retarding their metabolic rate, a condition known as quiescence, and or becoming ametabolic altogether, a phenomenon known as cryptobiosis or “suspended animation”. Within the scope of cryptobiosis is cryobiosis, which involves survival in low temperature conditions (i.e. cooling/freezing), and anhydrobiosis which involves survival without water.

Particularly within the last 25 years, studies on organisms capable of quiescence and cryptobiosis have greatly contributed to an understanding of how the biological integrity of function and structure, at the cellular and molecular level, is maintained in the presence of high osmotic or dehydration stress. Knowledge from these investigations have led to advancements in cryogenics, food stabilization, and drug delivery systems such as liposome technology.

Furthermore, it is now accepted that by studying organisms on earth that can inhabit inhospitable environments, ideas can be generated for modeling extraterrestrial life. For instance, on Jupiter’s moon, Europa, conditions such as severe dehydration, freezing, and a supersaturated brine ocean are constant factors a biological organism/entity would have to
content with. Similar stresses occur in specific habitats on earth (volcanic vents, desert lakes, etc.), and the organisms that are able to function in them, have begun to provide in model systems for studying these modes of survival at all levels of biological organization.

To date, most studies have focused on prokaryotic extremophiles and eukaryotic pseudocoelemates, particularly nematodes (Womersley, 1996). Many nematodes species demonstrated their ability to undergo cryobiosis in the presence of severe cooling/freezing dehydration stress (Womersley & Higa, 1998; Womersley et al. 1998).

Currently, the specific mechanisms and the orchestration of events necessary for and subsequent induction into cryobiosis, in the presence of severe stress, remain under speculation. *Panagrellus redivivus* is a new experimental model under development because this nematode lives in a temperate climate, entailing a natural freeze-drying process during overwintering, it was thought that cold-acclimation, prior to the initiation of a dehydration regime, would allow for the implementation of critical metabolic transitions necessary for cryobiotic and anhydrobiotic survival in the natural habitat.

**EXPERIMENTAL PROTOCOL**

*Panagrellus redivivus* was cultured on an autoclaved flour mixture of 50% all-purpose and 50% stone ground wheat flour. Cultures were maintained at 23°C for three to four weeks (allowing for adequate proliferation of the nematodes) before being placed in a variable-temperature incubator for cooling from room temperature to 15°C or 5°C through 1°C, 2°C daily incremental reductions in temperature prior to dehydration.

Nematodes were collected from the sides of the flasks, distributed in uniform aliquots into 5mL beakers, and placed in humidity chambers containing glycerol solutions for all humidities except 0% r.h. in which phosphorous pentoxide (P₂O₅) was used. These dehydration agents were selected due to their ability to remain stable and maintain a constant relative humidity under low temperature conditions. Each dehydration regime involved a grade-wise reduction in relative humidity from 86% r.h. to 0% r.h. at 24 hour intervals. Survival assessment was conducted after allowing samples to rehydrate at 100% r.h. for 24 hrs. by the addition of bulk water.

**RESULTS**

As seen in figure 1 below, nematode survival for the control experiment at 23°C (-86%, 72%, 62%, 55%, 0% r.h.) drastically declined at 55% r.h. (0% survival). Survival at 55% r.h. remained above 50%.

![Figure 1. Control P. redivivus Dehydration Experiment at 23°C](image1)

![Figure 2. Cold Experiment using P. redivivus Lowered Stepwise in 1°C, 2°C and 3°C Incrments from 23°C to 5°C and Dehydrated](image2)
As illustrated in figure 2 above, nematodes acclimated to 5C from 23C at 1C/day, 2C/day, and 3C/day increments did not survive at or lower than 55% r.h. following sequential dehydration (86%-0% r.h. regime). Throughout the regime, the 2C/day cold-acclimated nematodes demonstrated higher survival levels than the 1C/day and 3C/day cooled nematodes.

Figures 3, 4, and 5 present survival results for nematodes acclimated to 5C from 23C at an increment of 3C/day, and subsequently dehydrated along the following respective regimes: 86%, 72%, 62%, 55%, 0% r.h.; 72%, 62%, 55% r.h.; and direct exposure to 55% r.h. Survival for the dehydration regimes used in figure 3, 4, and 5 reached 0% at 55% r.h., 62% r.h., and 55% r.h., respectively.

In figure 6, nematode survival was plotted throughout an 86%, 72%, 62% r.h. regime following 15C acclimation from 23C using 3C/day temperature reductions. A drastic decrease in survival was observed at 72%; by 62% r.h., survival was at 0%.
DISCUSSION

The results indicate that nematodes acclimated to 5C from 23C are better suited for survival if a 2C/day rather than a 1C/day or 3C/day temperature reduction scheme is implemented prior to dehydration. The fact that an intermediate rate of cooling (2C/day) is optimal for survival suggests that the nematodes may draw upon valuable stores during lengthy cooling durations but may not be allotted enough time during rapid cooling to make necessary metabolic transitions for cryobiological and anhydrobiotic survival.

The terminal temperature point for acclimation to 5C was questioned in terms of extremity; thus an experiment adopting 15C as the final temperature was conducted (figure 6). However, the survival for the 15C acclimated nematodes declined prematurely. The observation that 5C rather than 15C acclimation supported survival may have implications with regard to analogous temperature conditions found in P. redivivus' natural habitat.

Using the 86%, 72%, 62%, 55%, 0% r.h. regime as a reference, successively more dehydration regimes were not conducive to survival (refer to figure 3, 4, and 5). This could indicate that implementation of metabolic transitions requisite for anhydrobiotic survival is facilitated by maintaining the nematodes at the higher relative humidities (i.e. 97%, 93%, 86% r.h., etc.) before exposure to subsequent lower humidities. Present research is aimed at testing these modified dehydration regimes.

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REFERENCES


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