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Suspended Animation - A Brief Review

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Abstract

Suspended animation (SA) is the temporary (short- or long-term) slowing or stopping of biological processes. Here, we will briefly review the current state of human long-term SA. There are two main fields where long-term SA constitutes an irreplaceable technology, namely manned deep space travel and cryonics, the cryopreservation of humans after clinical death with the aim to reanimate them in the future. In cryonics, much effort has been focused on the initial post-deanimation procedures collectively known as Standby, Stabilization and Transport (SST). Currently, shortly after death, bodies are stabilized and then transported to the cryopreservation organization, where cryoprotective perfusion takes place. Stabilization procedures range from covering the patient´s body with water ice, the simplest, to performing sophisticated procedures like emergency preservation and resuscitation (EPR) or deep hypothermic circulatory arrest (DHCA), in which the patient is taken to a condition of clinical death, characterized as electroencephalographic silence and cardiac arrest. The use of EPR and DHCA is unfeasible in the field. Experience shows that significant tissue damage occurs during the SST window. Bodies are finally placed in liquid nitrogen

(-196 °C) for long-term storage. Concerning reanimation, the use of the nematode Caenorhabditis elegans (C elegans) is proposed as a suitable animal model for studying reanimation. These worms tolerate cryopreservation in LN and can be efficiently reanimated without affecting memory integrity. Thus, C elegans may allow us to explore the mechanisms that allow the nervous system of these worms to tolerate storage in LN, keeping the integrity of its memory after reanimation.

Keywords: Spacetravel; Cryoprotection; CPA; Reanimation; EPR; DHCA; C elegans

Suspended Animation

Suspended Animation (SA) is the temporary (short- or long-term) slowing or stopping of biological processes. According to the purpose, it may be either hypometabolic or ametabolic in nature. It may be induced by either endogenous natural or artificial means. In its natural form it is usually reversed when environmental factors return to pre-SA conditions. If it is artificially induced it will require technologically mediated revival **[1]**. The present article focuses on human long-term SA. There are two main fields where long-term SA constitutes an irreplaceable technology, namely manned deep space travel and cryonics, the cryopreservation of humans after clinical death with the aim to reanimate them when medical technology achieves the cure for the condition that caused their death. An important difference concerning the application of SA to space flight and cryonics is that in the former SA will be used in young and extremely healthy individuals whereas in the latter, SA will be used on generally old terminal patients. Space travel- Concerning space travel, flying to distant planets or neighbor star systems, a journey likely to take years or even decades (Flight time for Voyager 2 to Neptune, 12 years **[2]**, would make it imperative to keep crews in biostasis in order to prevent aging and resource consumption. An additional benefit that SA will bring to space travel is the prevention of health problems posed by long-term exposure to interplanetary radiation and zero-gravity, as well as the psychological stresses and potential social dynamics disruption from confined space. An alternative to low-temperature biostasis is torpor, which also represents a state of slow metabolism that is usually induced by drugs and may be combined with milder hypothermia. Space agencies like the European Space Agency (ESA) and the US National Aeronautics and Space Administration (NASA) are both interested in implementing feasibility studies about SA for deep-space travel **[3-9]**.

Human cryopreservation (cryonics)

Clinical cryonics has been in practice for over 50 years **[10,11]** and involves two stages at which the temperature of the deanimated patient's body is lowered. The first stage involves short-term SA (hours) and takes place immediately after deanimation, when a standby team lowers body temperature to around 10 ºC, taking special care to keep the brain cool in order to lower oxygen consumption during cryopatient transportation from the site of deanimation to the facility where the body will be placed in long-term storage at Liquid Nitrogen (LN) temperature (-196 ºC). Concerning the first stage of clinical cryonics, over the years, research has been conducted with the aim of finding suitable procedures to submit large mammals to deep hypothermia by perfusion with cold asanguineous fluids that place them in a virtual SA for a few hours. The animal model typically used is the dog although

to a lesser extent, work on rabbits, lambs and pigs has also been published and will be briefly reviewed below. The cryopreservation process continues when the patient arrives at the long-term storage facility, where the body is intracarotideally perfused with a series of vitrification solutions in order to replace water in the intracellular and extracellular spaces by a solution that at LN temperatures will not form sharp needle-like crystals as water does, but an amorphous vitreous solid, thus the name vitrification **[12]**. At this point, the second stage of the vitrification process begins. The temperature of the vitrified body is gradually lowered until near nitrogen vapor temperature which is followed by immersion in LN at a temperature of -196 C. The body will be kept in long-term SA (years or decades) until a future when biomedical technology has achieved, besides the cure for the condition that caused death of the patient, a level of maturation that enables it to reanimate human bodies and return brain function and memory retrieval to a fully functional condition. While a substantial body of research has been carried out concerning cryopreservation procedures (see below), very scarce studies have been documented on reanimation of animal models after cryopreservation in LN. In contrast to cryopreservation procedures, cryonics organizations have virtually ignored another central procedure in cryonics, namely, reanimation. Almost no efforts have been made by these organizations to start reanimation research projects using suitable animal models. This is a paradoxical situation that places cryonics in a stand-alone position in the field of biomedical research. Stabilization of cryopatients for long distance transportation is not a biological requirement but an organizational modality that has led vitrification experts to spend years of work and significant financial resources on stabilization procedures that should be skipped altogether in favor of having standby teams able to perfuse patients, within minutes of being pronounced dead, with vitrification solutions. A vitrified patient is fully stabilized and can be safely transported at dry ice temperature to dedicated facilities for straight final storage in LN. This modality was employed for years by Cryonics-UK, a volunteer self-help cryonics group based in the UK **[13]**. When cryonics organizations describe their services, they provide copious details on the technology used to stabilize and prepare patients in the most convenient way to ensure the integrity of body structures during deep cooling **[14,15]**. But concerning reanimation, they only state to their customers that when future reanimation technology becomes sufficiently mature to reanimate frozen patients, the full process will be successfully completed. Yet, after over 50 years of cryonics practice, reanimation technology remains essentially unborn and reanimation technologies for mammals are nonexistent. This long-term lack of initiative makes it difficult to equate cryonics research to other biomedical areas of investigation. Let's take memory as an example of a typical biomedical field of research. The study of the basic cellular and molecular mechanisms underlying memory formation was begun by Nobel laureate Eric Kandel in a simple animal model, the sea slug Aplysia. Starting with this very simple animal model, Kandel could unravel how chemical signals trigger the formation and consolidation of new synapses, a process that is common to all neuronal systems, ranging from simple invertebrates to humans **[16]**. By the same token, the study of the nervous system of the model organism Caenorhabditis elegans (C elegans) could be instrumental to understanding what mechanisms

allow the nervous system of C elegans to tolerate storage in LN, keeping the integrity of its memory after reanimation [17]. In this short article we will review the progress on canine deep hypothermic SA and on the cryopreservation in LN and subsequent reanimation of the free living worm C elegans, of which more than twenty thousand different strains have been developed.

Hypothermic Suspened Animation in Large Mammals

Hypothermia has long been known to extend the window of organ viability and overall survival [18] a fact that cardiac surgeons [19,20], and neurosurgeons [21] use to their advantage regularly. Techniques for the controlled induction of hypothermia have been developed in animal models where hypothermia can be induced within minutes and tolerated for up to 3 hours without significant neurological sequelae [22]. Cardiopulmonary Resuscitation (CPR) has been one of the most paradigm-changing developments in modern medicine. When normothermic Cardiac Arrest (CA) occurs, brain damage starts after two or three minutes. After five minutes, global cerebral ischemia can lead to progressively worsening brain injury. By nine minutes of normothermic circulatory arrest, severe and irreversible brain damage is likely. After 10 minutes, the chances of survival are low [23]. If CPR is started within two minutes of CA, the possibility of preventing brain damage increases significantly. Widespread modern CPR education is responsible for survival to hospital discharge in up to 30% of witnessed out-of-hospital CA related to ventricular tachycardia or fibrillation, and outcomes have shown steady improvement over the years [24]. In contrast, the prognosis for cardiac arrest due to trauma is dismal. Even in the modern era of Advanced Trauma Life Support (ATLS), resuscitative thoracotomy, internal cardiac massage, and aortic crossclamping, overall survival remains less than 10% [25], Surgeons racing to control a major vascular injury in a dying patient are often outpaced by the speed of exsanguination and homeostatic imbalances. If rapid induction of hypothermia could provide more time, otherwise lethal injuries could be repaired. This approach is the basis for Emergency Preservation and Resuscitation (EPR) techniques for CA from trauma or Deep Hypothermic Circulatory Arrest (DHCA).

EPR

There are two types of hypothermic suspended animation used in medicine and in large experimental mammals, EPR and Deep Hypothermic Circulatory Arrest (DHCA). EPR is an emergency procedure used in cases of sudden cardiac arrest or imminent death from ischemia, such as the blood loss following a shooting or stabbing. In EPR, blood is partly or completely replaced by an ice-cold saline solution, and the patient is cooled into a suspended state where metabolism is slowed and brain activity ceases. This gives the surgeon perhaps 60 or 90 minutes to close the wounds before rewarming and recirculation procedures are applied. The concept of “suspended animation for delayed resuscitation.” was introduced in 1984 by Safar and Bellamy [26]. They defined EPR as induction of preservation of the organism within the first 5 min of CA (no-flow) for transport and surgical hemostasis during clinical death, to be followed by delayed resuscitation to survival without brain damage. Systematic studies of exsanguination CA were performed in dogs, with induction of preservation by aortic flush at 2 min after CA, of saline at 24°C, via a balloon-tipped catheter. By

lowering the temperature of the flushed saline to 2°C and progressively increasing the flush volume, starting the flush at 2 min after normothermic exsanguination CA, it was possible to decrease brain tympanic membrane temperature (Tty) to 34°C, which preserved brain viability during CA of 15 min [27] and 20 min [28], and to around 28°C, which preserved brain viability for 30 min [29]. In a systematic series of studies in dogs, the rapid induction of profound cerebral hypothermia (Tty 10°C) by aortic flush of cold saline immediately after the start of exsanguination CA, can achieve survival without functional or histologic brain damage, after CA no-flow of 60 or 90 min and possibly 120 min [30,31]. More recently, the first case of SA was reported in a patient who had a traumatic injury and entered CA. The patient was cooled to around 10°C and his blood replaced by ice-cold saline. The clinically dead patient was then taken to an operating theatre for a two-hour surgical procedure before having his blood restored and being progressively warmed to 37ºC [32].

DHCA

DHCA is similar to EPR in that hypothermia is induced. However, while EPR is an emergency procedure to prevent impending death and brain damage after sudden CA, DHCA induces hypothermia to aid preplanned surgery. DHCA is a surgical technique that induces deep medical hypothermia and involves cooling the body to temperatures between 20 °C to 18 °C then stopping blood circulation and brain function for up to one hour.33 It is used when blood circulation to or from the brain must be stopped because of delicate surgery within the brain, or because of surgery on large blood vessels that lead to or from the brain. The technique is used to provide a better visual field during surgery due to the cessation of blood flow. DHCA is a form of carefully managed clinical death. People (or experimental animals) who are to undergo DHCA for surgery are placed on Cardiopulmonary Bypass (CPB), a procedure that uses an external heart lung machine. In asanguineous procedures blood is removed and stored for later replacement. It is replaced by an acellular maintenance fluid [34]. Cooling continues until electrocerebral silence (flat line EEG) is attained [35,36]. The blood pump is then switched off, and the interval of circulatory arrest begins. In dogs cooled to temperatures around 2-5 ºC, nearly 5 hours of circulatory arrest was held before reversing the process [37]. According to this report, the neurological recovery of the animals was generally good. In human medicine, cooling does not go below 16ºC or so [35]. After surgery is completed during the period of cold circulatory arrest, these steps are reversed. Blood is restored to the circuit. The brain and heart naturally resume activity as warming proceeds. The first activity of the warming heart is sometimes ventricular fibrillation requiring cardioversion to reestablish a normal beating rhythm. Hypothermic perfusion is maintained for 10–20 minutes while on CPB before rewarming as to reduce the risk of increased intracranial pressure. Warming must be done carefully to avoid overshooting normal body temperature. Patients are completely rewarmed before discontinuing CPB. Ultra profound hypothermia (7ºC) coupled with complete blood replacement using protective hypothermic blood mannitol-based substitute solutions have allowed researchers to achieve reversible hypothermic circulatory arrest preserving the neurological function in dogs [37]. In order to induce very rapid hypothermia a variant has been introduced whereby

rapid hypothermia is attained by using cold perfluorochemical lung lavage (Liquid Ventilation (LV)) in dogs [38].

Cryopreservation and Reanimation in C. Elegans

The free living worm C. elegans is a simple nematode that can be easily isolated from high microbial substrates such as decaying fruits and stems, compost, and some invertebrates. Its life cycle, from an embryo to an adult, going through four larval feeding stages (L1, L2, L3, and L4), lasts 3 days (at 20°C). Nevertheless, this occurs as far as the animals are under favorable growth conditions. In nature, when the environment is stressful (lack of food, crowding), L1 larvae can enter an alternative stage known as dauer, in which they can live up to 4 months, and then continue their development until a reproductive hermaphrodite [39]. This small free-living worm has been especially useful for the study of developmental biology, cell biology, and neurobiology, thanks to its short but complex life cycle, the simplicity of its size (1 mm), and the transparency of its neural network. It contains 302 neurons [40] capable of producing highly plastic behavior, including learning (associative and non-associative) and memory. For all those reasons, C. elegans established itself as a standard model organism for a wide variety of investigations. The conservation of biological material at a very low temperature is a safe and inexpensive procedure that avoids DNA damage and strain contamination. There are two traditional ways to cryopreserve biological materials: slow freezing [41,42] and vitrification [43,44]. Both approaches have advantages and disadvantages, so their use is recommended depending on the context. Slow freezing requires a relatively low initial charge of cryoprotectant (generally a toxic agent), ice seeding, and a low, controlled cooling rate (generally 0.1 - 10°C/min) using appropriate equipment. In this strategy, extracellular ice is necessary (and therefore unavoidable) and the biological sample is subject to a large volume change. On the other hand, in vitrification, theoretically, there is no ice formation (neither intracellular nor extracellular). There is usually less volume change, and a high concentration of cryoprotectants. High cooling rates (the standard maneuver is cooling the sample in liquid nitrogen), and extremely high heating rates are necessary to avoid intracellular crystallization and/or recrystallization of small intracellular crystals during heating. The traditional way to cryopreserve C. elegans relies on slow freezing (which allows for ice formation) [45]. This method usually achieves survival rates of less than 25-35% for larval stages L1 and L2 [46]. Due to the ice formation previously described, individuals in the adult stage are not able to survive. Vitrification is an alternative procedure with higher recovery rate. For vitrification, very fine capillaries (thermoplastic polymeric capillaries which are sealed at both ends during vitrification and reheating, and opened after this step) [47] that maximize cooling and heating rates are used, allowing the concentration of Cryoprotectant Agents (CPA) to be drastically reduced inside the worm´s body. Then, the worms are hydrated and washed, to be finally transferred to an agar plate with a bed of E. coli and kept at 20°C. Survival rates obtained by this technique are close to 100% in the case of larvae (L1 - L4) – approximately 95% -. In the case of adults, these rates are close to 83%. These recovery rates make these worms a highly suitable animal model to study memory function after a vitrification-

reanimation sequence, a topic of paramount interest in SA research. Within this context, it is important to mention that it has been reported that when C elegans worms are trained in simple tasks, then vitrified and stored at -196 °C in liquid nitrogen, then reanimated, they fully remember what they have learned [17]. These results revealed that the animals fully tolerated the cryopreservation process, keeping the integrity of their memory and, consequently, of their nervous system structure. This animal model is not only convenient for basic studies on the neuroprotective mechanisms that allow the worms to tolerate ultra-deep hypothermia but it also lends itself as a superior bioassay to assess the toxicity of different CPA used in human cryonics. While other approaches assess the toxicity of cryoprotectants using ex vivo parameters like the K+/ Na+ ratio in slices of rabbit cortical kidney tissue [48] or cell viability in Bovine Pulmonary Artery Endothelial Cells (BPAEC) with glycerol (using PrestoBlue before and after CPA exposure) in automated settings [49,50], C. elegans allows to assess CPA toxicity directly measuring their effect on reanimation rates.

Concluding Remarks

There is a growing body of evidence showing that suspended animation is a goal already achieved in small organisms or biological samples, like embryos and ovarian tissue. The main challenge remains in the cooling technology, which in macroscopic organisms is still unable to attain the cooling speeds necessary to achieve successful cryopreservation. Deep freezing seems to be a mandatory requirement for SA, a fact that makes it difficult to fit SA units in the limited space available in current spaceships. In clinical SA, much effort has been focused on the initial post-deanimation procedures collectively known as Standby, Stabilization and Transport (SST). "Standby" means dispatching personnel and equipment to the patient's bedside, to provide information and expertise about his/her pre-mortem management and to prepare for transport. "Stabilization", means controlling the patient's condition, beginning at the time of legal death and terminating at the start of cryoprotective perfusion. "Transport", means that, according to the mainstream view in cryonics, it is almost always necessary to move the patient from the place where legal death occurred to an operating room maintained by the cryopreservation organization, where cryoprotective perfusion will take place. Stabilization involves different possible procedures, ranging from cooling the body by covering it with water ice, the simplest, to performing EPR or DHCA procedures as described above. Although EPR and DHCA are the procedures that offer the best protective results, they are highly sophisticated and, in the case of DHCA, usually require a surgery room, something that cannot be fitted in an ambulance or other types of transport vehicle. In practice, none of current SST services can prevent a significant amount of tissue damage to occur during transportation. This scenario suggests that the best strategy would be that the standby team perform the cryoprotective perfusion on site. Cryoprotective perfusion immediately after death would avoid the damage that inevitably occurs during SST procedures. In technical terms, pre-mortem perfusion would be even more effective but this is at present, legally unacceptable. It seems conceivable that in a near future, SA technology will make advances that will bring to medical technology the capability to offer currently incurable patients a second chance at life in the future.

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