

# Cryonics, a Comprehensive Approach. The use of Higher Temperatures and its Viability

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## Abstract

Many papers study cryonics, its feasibility and effectiveness as the only currently available method for preserving life beyond the declaration of clinical death. The estimated resuscitation times of each patient and how this affects the selection of the appropriate temperature for cryopreservation is introduced. The analysis of enzymatic reaction times proves that it is not necessary to cryopreserve at  $-196^{\circ}\text{C}$  but at a higher temperatures, This is the main contribution of this work.

The scope of the analysis covers the use of which substances, cryoprotectants, as well as inhibitors, should be used as well as the enzyme reaction times that take place at different temperatures.

Not only the technical details are analyzed, but also the legal perspective. Cryopreserved subjects are considered "patients" and not lifeless bodies. It is explained why this is necessary and why it should be communicated at a social level through state policies, as well as the absolute necessity to apply an immediate legal and juridical modification that allows starting the procedures without delay before the metabolic arrest of the patient, and why the World Health Organization already guarantees this in its constitution.

The aim of this paper is to confirm the cryonics method as a current solution to pause biological time and give the patient a chance, which by right, to preserve his or her life.

**Keywords:** cryonics; cryopreservation; cryobiology; cryoprotective agents; intracellular ice formation

## Introduction

According to the Cambridge dictionary "cryonics" in humans is defined as: "the process of storing a dead body by freezing it until science has advanced to such a degree that it is able to bring that person back to life"[1]. This definition has the problem that refers to a lifeless body, and not to a suspended life body, with its implications, that is the potential to live. It also does not refer to cryonics as a branch of physics, that deals with very low temperatures that do not occur naturally on Earth, as well as the reactions of matter exposed to these temperatures.

It is also defined as the science and technology of temperatures below 120k or -153.15° Celsius, which is the boiling point of major atmospheric gases such as methane [2].

A cryogenic experiment consists on cooling a substance and keeping it cold at a certain temperature, typically under -153.15°C, but in reality, this includes higher temperatures as well.

The device used for this is called a cryostat, which is a device able to control extremely low temperatures and prevent heat transfer from the outside to the sample by a combination of insulation or cooling techniques. The storage and transport tubes of cryogenic liquids are called Dewar [3].

To reach such temperatures two methods are used, one is by the use of cryogenic liquids and the other is by means of mechanical coolers.

The term liquid cryogen refers to liquid Nitrogen, at a temperature of -196°C, or Helium, at -269°C.

The most widely used technique is liquid cryogenic cooling. Heat is used to warm the evaporated gas at the boiling point to room temperature. The heat required for this process is given by the change in enthalpy, which is a thermodynamic property that measures the amount of total energy contained in a system. When the system has absorbed heat there is a positive enthalpy change. In cryonics enthalpy change is forced in the dewar tubes at the neck, which allows cooling in this radiation zone to maintain the desired temperature.

In cryonics temperatures for humans are never higher than -196°C, so liquid nitrogen is the option of choice due to its low cost compared to helium [3].

The rest of this section introduces regulatory concerns and technical issues, section 2 explains biological considerations, section 3 is a background summary of the field of work, section 4 presents materials and methods, section 5 depicts the proposal, section 6 is a simulation and its results, section 7 are discussion and analysis, section 8 are conclusion and future work.

### ***Cryonics and public understanding***

Cryonics involves the preservation of a body that keeps its structures fully intact, not functioning at that time but is able to be reanimated. In this context we analyze whether we are really treating patients or dead, lifeless persons [4].

Death is currently associated with the loss of brain functions. It should be defined as the irreversible loss of life in an absolute and definitive manner, when the brain structure is destroyed. This is what medicine defines the arrest of heartbeat and respiration without damage to brain cells as “clinical death”. In this condition the brain structures are in such a condition that can be reanimated. The actual condition of the patient is metabolic arrest with today’s terminology applied, termed “clinical death”, when simply the more appropriate term “ametabolic coma” should be used [4]. It is for this reason that categorical death cannot be declared without access to the tools that the near future will offer, such as nanotechnology. If new technologies are used and reanimation is not possible with these tools, it would be then possible to declare an absolute death.

Emphasis is placed in this work, on the correct definition of what cryonics means, as this could improve the timing and procedures of the system itself, making the execution times more efficient.

Today, scientists working in cryonics must deal with the legal artifice of having to declare clinical death first, resulting in a longer waiting time before starting the procedure. It should be made clear that cryonics is trying to save the lives of patients, not that it is trying to save patients who have already died. Having to wait for the patient to be declared clinically dead is simply a legal regulation and the scientists are forced to wait for the patient to be declared deceased in order to start the procedures. This situation is of little help in preserving the patient’s life.

Cryonics is by no means an alternative to cremation or burial of the body. In the above cases the body decomposes, therefore cryonics can best be defined as a life-saving method [4].

### ***Cryogenics in the economic development of a country***

The change in perspective from the very concept of the definition of cryogenics makes the society in general to gain interest in it. It also makes that state policies be established where this technology is promoted. This situation is such that the use of cryogenics for a country is synonymous of progress. Certain countries annually increase their cryoliquid consumption by 12-15% [25], which is an incentive for the application and development of these technologies in a nation.

Cryoliquids are used in different procedures and awakening their economic interest helps to position a country for its scientific and technical potential. It is not only about technological and scientific development, but also brings significant economic benefit to the country that successfully generates it [26].

### ***Technical aspects. The basics***

It is theoretically possible to preserve at very low temperatures a human body which is potentially capable of living again. It should be only mentioned "theoretically" because it has not yet been successfully performed in humans. This can be done by means of the vitrification method, a topic that will be developed further in this paper. By using such temperatures the neuronal structure, which gives rise to the human mind, can be preserved.

When blood flow is stopped in the body for 6 minutes or longer and blood reperfusion is performed, the damage caused is mainly due to the vessels corruption and not due to the neurons. This is important to note because apoptosis of neurons, takes hours to complete. It is important to start procedures as early as possible, ideally within the first minute of clinical death, however its useful to start the procedures later on and still be a viable method.

A process that could be technically faster to execute if legal conditions were adequate to implement it, is delayed. This happens only because the procedure can be started, as mentioned, once the legal death takes place [5].

The underlying fundamentals of the application of cryonics on humans are that the low temperature reduces metabolism and that if it is low enough it can stop chemical changes. One of the problems to be addressed, is ice formation. It can be reduced or eliminated by the use of vitrifying mixtures. The fact that a patient is declared legally dead is a reversible process, since death is not an event that occurs at a given moment, but is a process that takes many hours, much longer than the 6 minutes that are commonly considered to declare it [5].

### ***Technical aspects. The procedure***

After legal death the patient is placed in an ice water bath, since initially a quick lowering the temperature can greatly increase the time the body can be kept without blood flow before any damage occurs. Mechanical cardiopulmonary support is then applied, which in turn accelerates the cooling effect by transferring heat from the blood. The cooling process then consists of convection, which is a combination of conduction and fluid movement. As the fluid moves, it carries heat away from the subject, moving from 37°C to 10°C by the rapid circulation of water. The faster the water circulation and thermal conductivity, the greater the temperature variation.

Note that cooling occurs faster in a flow of water than in still water, so it can be stated that cooling is much faster in this order, from slower to faster: air, still water, moving water flow (according to Newton's law of cooling). It is also important to note that vitrification starts below 10°C. After this circulation, the blood and a large percentage of water are removed and replaced by a cryoprotectant that prevents the ice formation. The body is then cooled to -120°C and then brought to -196°C. With the current liquid nitrogen method, patients are stored in Dewar tubes at this temperature. Dewar tubes are inexpensive and not dependent in terms of safety on power outages or power failures. In this way, it is preserved in a cryostat for later resuscitation and rejuvenation if necessary [5].

Cardiopulmonary resuscitation (CPR) should not be performed since the patient has already been declared clinically dead, only cardiopulmonary support (CPS) should be performed. To prevent resuscitation from occurring, a sedative that prevents resuscitation is applied (Propofol 2,6-diisopropylphenol), which in addition is also neuroprotective and prevents neuronal apoptosis that could occur due to ischemia or reperfusion. Heparin is also given to prevent blood clotting and streptokinase which is a thrombolytic and is used to dissolve blood clots. A buffer (THAM) is also used to keep the extra and intracellular pH constant so that it does not produce CO<sub>2</sub>. Epinephrine is used as an aggregate to the cryoprotectant to maintain blood pressure, to which sometimes vasopressin is added [5].

Regarding the brain and its vitrification, it is achieved by increasing the exposure to cryoprotectants for a longer time at the expense of increased toxicity. Since it is not the same as for vitrification of smaller organs or other tissues, therefore a higher concentration of the same must be used.

At -196°C there is a possibility of fracture due to thermal stresses and for this reason it is recommended further on in this work to reach a minimum temperature of -130°C, since even at this temperature the patient is preserved in a solid state and the total vitrification occurs below -120°C. This method is safer and has not yet been implemented [5].

Even with all these measures there is a possibility of intracellular or extracellular crystallization that can damage tissues, although this possibility is present, what is attempted with these procedures is the reduction of damage. The damage occurs when the cells are cooled, in this situation the water osmotically leaves the cell and forms extracellular ice in the form of crystals. This is due to the fact that it is not possible to freeze the whole body at the same time. Since it is not possible to do this, the solution is left with different concentrations of electrolytes, and the area with the highest concentration of electrolytes is the most toxic. On the other hand, the extracellular ice could destroy neighbouring cells by crushing [6].

In an attempt to reduce and minimize this ice formation, cryonics patients are perfused with cryoprotectants, which are compounds that prevent ice formation, usually glycerol. By 2007 the largest cryonics organizations claim to have prevented brain icing by using a vitrification solution.

Prior to vitrification, cryoprotectants produced a combination of structural damage and toxicity that would be very difficult to repair. With vitrification this is solved. High concentrations of cryoprotectant cool rapidly without ice formation. The solution of water and cryoprotectant passing below -120°C solidifies and even the cytoplasm remains intact, so that biological time is stopped. This is the major difference between freezing and vitrification, there is no structural damage [7]. Further discussion on cryoprotectants is provided in section 2.2.

## **Biological considerations**

### ***A word on ischemia***

We have previously discussed damage to blood vessels as well as damage to neurons, in both cases due to ischemia, which is the absence of blood flow. Within the ischemia the patient may suffer is considered “warm ischemia” which occurs when there is cardiac arrest in normal conditions and temperatures at the moment of legal death and there is no immediate cooling. “Cold ischemia” occurs when the patient is on ice for longer than expected without the supply of cryoprotectant, which leads to damage to the blood vessels and causes them to leak, resulting in local edema and a dysfunctional blood vessel. By having dysfunctional vessels the tissue is compromised with cellular damage and this is very common also in organ transplantation, when the organs are cooled to prolong their viability before transplantation.

Unfortunately due to the non-authorization of the procedure without the presentation of clinical death, minimal ischemic damage will always be present, this is due to legal terms and this means that there is always some pre-mortem ischemic damage, but always the most damage is post-mortem, so by reducing the time as much as possible from the declaration of clinical death the less damage will be caused to the patient.

The less ischemic damage, the greater the possibility of successful reanimation. As some damage is inevitable, emphasis is placed on preserving the vessels supplying neurons and their connections. Consequently, if circulation is resumed immediately post mortem after a few minutes, the tissues benefit from this, otherwise if it takes longer, the vessels are more damaged even without providing restoration of circulation and this causes a greater amount of free radicals and more damage due to reperfusion. The damage is not to produced by the vessels themselves, but the blood vessels must be in good condition to transport the cryoprotective vitrification solution to the brain. If this were to fail, ice would form in the brain, which some scientists do not consider to be a major problem since nanotechnology could reconstruct it in the future [8].

With respect to ischemia, as mentioned above, the 6 minutes after cardiac arrest and cessation of blood flow are not determinative of neurological damage, which is a common assertion in the medical field today. This statement is incorrect, since what does happen is that a process of apoptosis of the brain structure begins, but this process takes many hours to complete and brain cells are mostly preserved [9]. This assertion will be supported later in this paper with further evidence from section 1.7 which corresponds to animal tests.

It is consequently understood that in a suboptimal scenario, in which there really is neuronal damage due to ischemia, stem cell transplantation has the potential to restore normal functions. This type of procedure increases the amount of tolerable ischemia, but it should be taken into consideration that as little damage as possible will always be preferable before the start of cryopreservation [10].

Companies that perform this procedure begin after the patient has been declared dead, within two minutes and not exceeding 15 minutes [16].

This is the moment when the cooling of the body begins, which is the most important step to avoid ischemic damage and it is important to note that every 10°C reduction in temperature decreases the metabolic rate by 1/3 to 1/2 [5].

This temperature reduction causes hypothermia, which depresses protease activity and thus protects the blood-brain barrier, especially the endothelial zone and the basal lamina surrounded by extracellular matrix and type 4 collagen, otherwise proteolytic enzymes rapidly digest the basal lamina exposing the brain parenchyma to blood flow, with its consequent deterioration [17].

### ***Cryoprotectants***

As mentioned above, cryoprotectants are used to prevent or eliminate the ice formation when cooling the organism, but this has its limits, and that limit is the toxicity caused by high doses of cryoprotectants, which is an impediment to keep under control in the vitrification process [11].

In order to minimize damage, it is necessary to determine which macromolecules and organelles are damaged by excess cryoprotectants and what is the process by which this happens. The method would be to expose different cells to different cryoprotectants and see what results and what damage occurs. DNA, proteins, mitochondria, etc. should be evaluated to determine the molecular mechanism causing the damage [12].

If, for example, during the previously mentioned experiment to identify the molecular mechanism causing the damage, we detect that the toxicity of the cryoprotectants causes caspases, proteases or kinases to be activated leading to a process of apoptosis, then we could intervene to reverse these processes. This is something feasible and is done after what is called “post-thaw-culture” (cell culture of samples that have been thawed, common in the field of cryopreservation). In these cases, apoptosis and necrosis are evident within 6 to 24 hours after post-thaw-culture, but by incorporating apoptosis and necrosis inhibitors (zVAD-fmk, p38 MAPK inhibitor, ROCK inhibitor, etc.), caspases, proteases and kinases are inhibited, thus preserving the integrity of tissues and cells [13].

## ***Cremation and Burial***

Having evaluated public and scientific opinion and having discussed the methods and techniques of cryonics, as well as its benefits and the fact that death is not an event that occurs at a precise moment in time, but takes hours to be completed, it is not possible to continue with the current understanding of cryonics and its use as another means of disposing of the body post-mortem. It is not logically correct to compare it to a burial or cremation of the body, as these other methods make death certain and irreversible, when the process of death is not [14]. The approach to understand this must start from the very understanding of what human life is and why it is wrong to speak of death, when in no way should it be considered as an irreversible process. In the case of clinical death there is never death, since this is a reversible process and only the metabolic function ceases. For this same reason we refer to cryopreserved subjects as patients, although legally they can no longer be considered as such.

When clinical death is declared, the patient loses his or her rights and is no longer considered a “person/individual” but a “thing”, therefore in the case of reanimation the law should stipulate how his or her future legal consideration would be. This is an ethical and legal debate, but with the right technology and without legal impediments on cloning and epigenetics, a copy of the damaged tissue could be made and replaced if necessary. This would lead to a legal redefinition of the reanimated person [15].

## **Background**

### ***Successful cases in animals***

Conducting a series of animal reanimation experiments shows more than satisfactory results. This is exemplified by 25 rats exposed to a temperature range of 0-1° C, successfully undergoing complete reanimation using microwave diathermy, which is a technique used in physical medicine to generate heat in deep tissues of the body, this generates heat at the cellular level. In this way it was possible to obtain 80-100 % successful reanimation rates.

In 1955 one of the rats was successfully cooled and reanimated 10 consecutive times at intervals of 2-10 days at 0-0.5° C (The apparatus used was a “magnetron microwave generator” powered by a 500W continuous wave magnetron at a frequency of 3000Mc/s designed to heat frozen blood samples) [18].

In 1957, rats were refrigerated at 2-3°C and kept in this state for periods of up to one hour without any activity, neither cardiac nor respiratory. This is due to the accumulation of metabolic CO<sub>2</sub>. The animals are artificially ventilated during resuscitation, otherwise they do not recover [19].

Regarding the cerebral cortex of rats and its consequent ischemia due to blood flow interruption, only 15% of the neurons show necrosis after 6 hours. The majority of neurons do not show necrosis until 12 hours after cessation of blood flow, this represents about 65% of neurons [9].

In 1966, glycerol was applied as a blood substitute to reduce ice formation in cat brains, refrigerated at -20°C. After 45 days without circulation, brains with normal encephalographic activity were revived [20].

In 1976, dogs were subjected to cardiac arrest for 12 minutes, then blood pressure was raised and norepinephrine, heparin, and hemodilution with dextran 40 were applied. This demonstrates that neurons are not destroyed after a few minutes of ischemia and what prevents the dog from surviving is resistance to blood recirculation [21].

Subsequently, in 2003, hypothermia was performed in dogs, reaching a tympanic temperature of 10°C. Under these conditions, 100% of the dogs show no neurological deterioration before 90 minutes of cardiac arrest. When prolonged to 120 minutes only 2 of 7 dogs show no neurological damage [22].

In addition, the vitrification mixture M22 preserves brain structure in rabbits without icing [24].

In humans, hypothermic cardiac arrest is performed for aortic surgery for more than one hour without neurological deficit. The temperature used is 16°C-24°C and the patients reach an electroencephalographic index of zero, which means total electrocerebral silence, after which brain activity returns with intact personality [23].

## **Materials and Methods: this section explains some of the hypotheses, general objectives and specific objectives**

### ***Working Hypotheses***

This section describes the hypotheses considered to determine the type of work to cover.

H1. Public knowledge about cryonics and its meaning is incorrect.

H2. Cryonics is a viable and useful technique and treatment to prolong the time of biological viability of a patient with metabolic cessation, avoiding cellular and organic deterioration.

H3. The time used to cryopreserve the patient is dependent on the time needed to find the cure and design the resuscitation technology.

H4. The times of metabolic and apoptotic enzymatic reactions of the patient determine the temperature used for cryopreservation.

H5. The use of a temperature higher than -196°C is feasible for cryopreservation in humans.

H6. H5 is dependent on H4 and H3.

H7. The efficiency of cryopreservation methods can be improved by improving cryopreservation implementation times through legal regulation.

### ***General Objective***

This work aims to evaluate both the understanding of cryonics from the social and scientific perspective to the analysis of the techniques and the legal approach. It evaluates the feasibility of successfully preserving a human body through the use of a conventional cryogenic system and how to implement an optimized cryogenic method.

The process starts from the very conception of what the definition of death means, for the general public as well as for the scientific community.

To evaluate in which conditions the body should be in the moment before the metabolic cessation and also during cryogenic storage.

### ***Specific Objectives***

1. To determine the public acceptance of the cryonics method, with the current public knowledge, by means of a randomized survey.
2. To determine the feasibility of cryopreservation as a method of preserving a human body.
3. To estimate the preservation times of patients prior to reanimation.
4. To estimate the enzymatic times of metabolism at different temperatures for cryopreservation.
5. To estimate the appropriate temperature for cryopreservation.
6. Determine the methods to be implemented for eventual improvement in cryopreservation.

### ***Criteria for validity***

In order to determine the confidence level of the results, certain standards of determination are being applied to the bibliographic material. The material focuses on cryonics and covers at least one of the accompanying topics:

- Cryonics, terminology and its public and scientific understanding.
- Feasibility of cryonics as a method

- Relationship of cryopreservation temperature to vitrification/crystallization/fracturing of structures.
- Ischemic damage in cryonics patients.
- Temperature adjustment considering enzymatic reaction times.
- Cryoprotectants, inhibitors and facilitators of apoptosis.
- Relationship of ischemia/low temperature experiments in animals.
- Expected times to find a cure for various diseases.
- Cryonics, legality and human rights in this topic.

Any other material not related to these topics, although potentially very solid and aligned with the current objectives, is being discarded. This is because the section is intended to be rooted solely in the understanding of cryonics, its feasibility, technical analysis and related legal human rights.

## Proposal

The main contribution of this section is to provide information in order to improve the understanding of cryonics and cryopreservation times in relation to the timing of enzymatic reactions in the human body.

As a general approach, the bibliographical coverage is:

- The technical procedure of cryonics
- The response of the body, tissues and cells to low temperatures.
- Metabolic rate regulated by temperature.
- To explore animal experiments and their results.
- Legality and general public understanding of cryonics.

## Materials and Methods

The study to collect data on the public acceptance of the method is carried out with data collected from face-to-face surveys in the public street, as well as online surveys with mixed audiences from different countries to cover an international opinion.

The survey consists of taking the following data from the participants: age and gender.

The survey consists of the following statements:

- A) Would you cryopreserve your body under the possibility of being able to bring you back to life in the future?
- B) Do you agree with the cryopreservation method?

The answers are as follows: YES/NO. From these data we concluded in percentages, both the level of acceptance to be cryopreserved and the social acceptance of the method.

Information from previous publications and works in this field is analyzed.

The calculations of the enzymatic times of metabolism are made based on the enzyme lactate dehydrogenase as a representative model. For this purpose, we calculate the different reaction times of the enzyme according to the temperature variation using the Arrhenius formula.

## Survey & Simulation test results

### *Survey results*

We carried out a survey to a total of 500 people in the public street, in person and randomly, of which:



The gender that voted was 48% female 49% male and 3% non-binary.

The age of the respondents was between 18 and 72 years old and the following results were obtained.

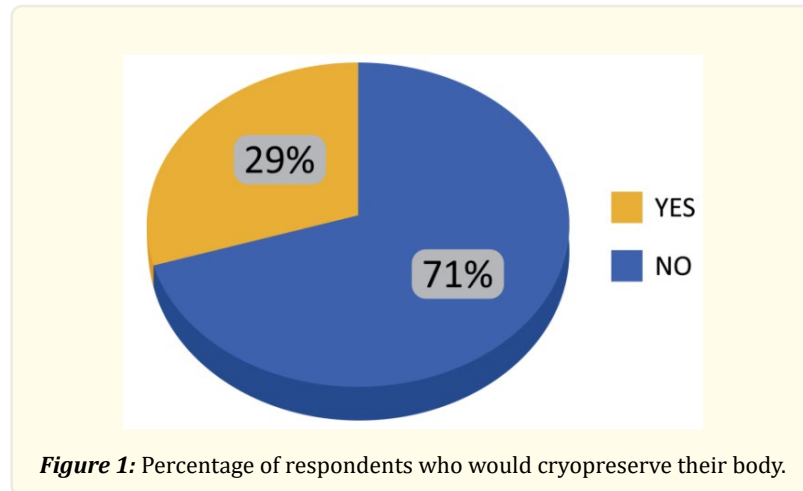


Figure 1 shows the results to the following question:

***Would you cryopreserve your body under the possibility of being able to bring you back to life in the future?***

71% responded that they would not cryopreserve their body and 29% responded that they would.

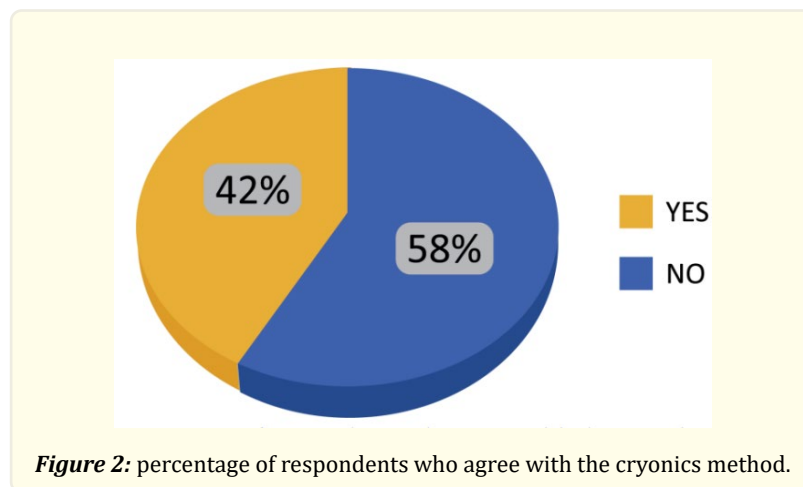


Figure 2 shows the results to the following question:

***Do you agree with the cryopreservation method?***

58% responded that they do not agree with the method and 42% responded that they do agree.

**Simulation test of LDH enzyme times results**

The following tables were designed with the use of the results obtained from the calculations of the enzymatic times of the enzyme lactate dehydrogenase, which was taken as representative for the metabolism.

Data	Relation K1/K2 17,85383464			
Temperature 1 Celsius	37			
Temperature 2 Celsius	0			
Activation energy (J/mol)	54810			
K1 (minutes)	6			
K2	107,1230078	1,785383464	0,074390978	0,000203811
	Minutes	Hours	Days	Years

**Table 1:** Calculations of LDH enzyme and its reaction times at 37°C and 0°C.

Table 1 shows the results obtained using the enzyme activation energy, which is 54810 J/mol. The table is designed to calculate the variations in the reaction rates with the Arrhenius formula. The table shows the relation between the reaction times of the enzyme at 37°C under normal conditions and at 0°C with decreasing temperature. It shows a ratio of 17.85383464 times greater with respect to the time required to perform the reaction at 0°C than under normal conditions. That is, an event that would take 6 minutes at 37°C would take 107.1230078 minutes at 0°C. We take 6 minutes as a reference for the time in which the ischemia is performed.

Temperature	Relative rate at 37°C	Relative time to 6 min at 37°C
37°C	1	6 min
27°C	2,03	12 min
17°C	4,33	26 min
7°C	9,76	58 min
0°C	17,85	107 min
-20°C	120,44	12 hr
-50°C	4010	16,7 d
-80°C	400000	4,5 yr
-90°C	2,6x10 <sup>6</sup>	29 yr
-95°C	7x10 <sup>6</sup>	80,6 yr
-100°C	2x10 <sup>7</sup>	235 yr
-105°C	6,4x10 <sup>7</sup>	731 yr
-110°C	2,1x10 <sup>8</sup>	2436 yr
-115°C	7,7x10 <sup>8</sup>	8760 yr
-120°C	3x10 <sup>9</sup>	34253 yr
-125°C	1,3x10 <sup>10</sup>	146858 yr
-196°C	9x10 <sup>27</sup>	1x10 <sup>23</sup> yr

**Table 2:** Results of LDH reaction times from 37°C to -196°C.

Table 2 shows the data extrapolated using the Arrhenius formula. The results cover temperatures ranging from 37°C to -196°C.

It can be seen that with decreasing temperature the reaction time of the LDH enzyme increases exponentially.

The table shows the relationship between the reaction times of the enzyme from 37°C under normal conditions to -196°C, showing a dramatic change in the ratio of up to  $9 \times 10^{27}$  times greater than the time required to perform the reaction at 37°C.

The 6 minutes refers to the biochemical reaction of ischemia produced in 6 minutes at 37°C under normal conditions. This biochemical reaction would take 80.6 years at -95°C and 235 years at -100°C. At the proposed temperature of -125°C it would take 146858 years for this reaction to take place.

## Discussion

### *Feasibility and efficiency of the procedure*

Through this research it was determined that cryonics is indeed a viable method for the subsequent reanimation of a human body and that it can effectively increase life expectancy, by means of the time given in years to solve and find a cure for the pathology in question that caused the death in the first place, consequently allowing a prolongation of human life as we know it.

The research was carried out starting with surveys, collecting data to know the social acceptance of both the acceptance of the method individually and the acceptance of the cryopreservation method per se (see Fig. 1, 2).

Through these data we determined that cryonics does indeed have a low acceptance rate in both cases, both for potential personal use and for acceptance of the cryopreservation method as a technology.

The result of the low acceptance is attributed to misinformation among the general public and the misconception and misunderstanding of what cryonics itself represents as a life-saving possibility. There is an understanding that cryonics involves biological death. While the procedure is performed once clinical death is declared, this does not mean that biological death occurs.

Since there is no brain death, individuals are referred to as patients, in this case cryopreserved patients. This is the major point of divergence with the public thinking and understanding of cryonics, and the reason why the survey yields such results. By implementing state policies of communication and information and clarifying the concept itself, public opinion would change drastically and the method would become more widely accepted.

Even as mentioned above, the Cambridge dictionary itself defines cryonics as the preservation of a dead body [1]. This is an erroneous definition, it must be understood that this is only a gimmick used to overcome legal obstacles, which otherwise would not allow the procedure to be carried out.

The efficiency of the procedure is now being discussed and public and scientific opinion is divided.

Mainly it is debated whether the patient can be reanimated after the procedure. This is discussed simply because it has not yet been performed in humans, but there are cases in other animals reaching higher temperatures, between -20°C and 10°C [18, 20, 22]. Although the temperatures are not close to -153.15° Celcius, which is considered cryogenic temperature by definition, the efficiency of reanimating the animals in case of hypothermia was demonstrated, as it was done with humans for aortic surgery procedures in temperatures between -24°C and 16°C. The encephalogram index was zero and then returned to normal with brain functions in perfect conditions [23].

The reanimation process is feasible, the drawback is the temperature variation reaching such low temperatures and the time during which the patient remains at those temperatures without biological alterations or rupture of structures. Taking this into account, the optimization of the appropriate temperature and the time during which the patient is in this state could be the solution.

Understanding that this is a technological drawback to be solved and that the patient, even without vital signs, is not dead, but is in a state of suspension and metabolic suspension with its structures fully intact, would drastically change the perception of public opinion.

The declaration of clinical death, as mentioned above, is simply a gimmick to be able to perform the procedure. In the absence of adequate legislation that would make it possible to carry out the method within the framework of legality, it is decided to carry it out by declaring clinical death. This has a direct impact on the perception of death and the perception that what is really being preserved is not a patient but a dead body, which is completely erroneous, since the loss of life is totally reversible with the use of the appropriate technology. This is why it is proposed the conceptual and terminology change from “clinical death” to “ametabolic coma”.

By establishing the appropriate terminology, treatment times would be much more effective. Declaring an “ametabolic coma” would avoid unnecessary waiting and cryopreservation procedures could be started immediately, avoiding or reducing any deterioration under normal conditions until eventual reanimation. For this reason cryonics should never be considered as the preservation of a dead body, but on the contrary, as a way to save lives [4].

Interfering with cryopreservation methods that can be performed in a timely manner hinders the potential life-saving process. This should be very clear, not only that it hinders it, but that not being allowed to perform the procedure irreversibly ends the patient's life. This is a legal problem for the state, since it should guarantee the safety and well-being of its citizens, and for this reason the state is urged to promote laws that guarantee the health and well-being of its citizens by passing laws for the immediate implementation of cryonics and a legal re-definition.

The greatest efficiency is time-dependent, but when the performing times are not optimal the procedure must be carried out anyway at the time clinical death is declared, since there is no neuronal death, but rather the damage of the vessels is mainly affected. In fact, a body whose heart has stopped beating takes many hours to undergo neuronal apoptosis and should not be declared dead, since the non-functional element in this situation would be the vessels, so that a patient declared legally dead is in a totally reversible state [5].

Initially, after declaring clinical death, the body is brought from 37°C to 10°C and blood is pumped mechanically, which accelerates the cooling process. During this process, care is taken not to reanimate the patient that has been declared clinically dead. To this end, a sedative is applied to avoid this and the patient is brought to a temperature of -196°C. To reach this temperature, higher temperatures such as the vitrification state at -120°C are first reached. At -196°C the bodies are preserved in Dewar tubes, where there is a possibility of fracture due to thermal stresses at that temperature [5].

Even with the current procedure there are risks of ice formation, since when mammalian cells are cooled, the water osmotically leaves the cell and forms extracellular ice in the form of crystals. Since not everything freezes at the same time, neither the intracellular nor the extracellular components, the extracellular ice would crush the remaining unfrozen cells [6].

It is to reduce this ice formation that cryoprotectants such as glycerol are used. In 2007 by vitrification process and use of suitable cryoprotectants this ice formation was avoided by using high concentrations of cryoprotectant, which was rapidly cooled without ice formation below -120°C. It was seen that even the cytoplasm solidified and remained structurally undamaged [7].

### ***The unnecessary use of such low temperatures***

For this reason, it is proposed in this work to use temperatures above -196°C, which corresponds to the temperature of liquid nitrogen, in order to avoid these stresses that could produce fractures in the structures. The proposed temperature for patient preservation is -125°C,-130°C, once the vitrification point has been reached. This is because a lower temperature could be counterproductive because of the risk of fractures and in turn does not provide additional benefits for the reduction of metabolism and arrest of metabolic processes as will be seen later in an analysis of enzymatic reactions. This marks a difference of about 70°C above the temperature of the liquid nitrogen currently in use.

As mentioned, at  $-120^{\circ}\text{C}$  vitrification of the body occurs, which with the use of cryoprotectants can preserve the integrity of the tissues in an almost complete arrest of cellular metabolism.

The selection of  $-125^{\circ}\text{C}$  is not only due to the preservation of cellular structures, but also to an analysis of metabolic reactions at that temperature, which are so close to zero that it is as if the body were in an ametabolic state [5].

Catalyzing the reaction of an enzyme requires a certain time and this time is dependent on the temperature at which the enzyme is working. To perform this calculation, the simplified Arrhenius formula used in a scientific work of 2008 [5] was used to calculate the enzymatic reaction on the enzyme LDH (lactate dehydrogenase) of rabbit muscle, selected to be representative of the metabolism. With these results the researchers made in the same work a table that included the reaction times of the enzyme at  $0^{\circ}\text{C}$ ,  $-80^{\circ}\text{C}$ ,  $-120^{\circ}\text{C}$  and  $-196^{\circ}\text{C}$ .

In this work we verified some of the calculations made and corrected others that were inaccurate in the table of the previous work of 2008 [5]. In one of the cases the reaction time presented for the LDH enzyme at a temperature of  $-120^{\circ}\text{C}$  was 34000 years, when in reality it is 34253 years (See Table 2) and this represents a big difference when selecting the temperature taking into account the reaction times.

We verified the data using the enzyme activation energy of 13100 cal/mol [27] or its calculated equivalent which corresponds to 54810 j/mol (See Table 1). However, the data presented in their work, apart from a possible inaccuracy, is not useful when considering the possible cryopreservation times to be used in patients at  $-125^{\circ}\text{C}$  as proposed by this work. The reasons for this are detailed hereunder:

A process that takes 6 minutes at  $37^{\circ}\text{C}$  can take 34253 years at  $-120^{\circ}$  and 146858 years at  $-125^{\circ}\text{C}$  (See Table 2), which is the temperature proposed to preserve the patient's body. Therefore, it is to be concluded that this temperature is more than sufficient for the preservation of the body over time.

The estimated preservation time must be taken into account when evaluating the temperature and preservation of the biological structures. The question then arises as to how long the body should be in the ametabolic state.

### ***Time before reanimation***

First of all, it must be kept in mind that prior to decrionization, a cure for the disease must be found and therefore this will be the determinant of the total time, in addition to the development of the technology to resuscitate the patient's body. If a cure is not found, no attempt should be made to reanimate the patient. Taking this into account, the most important time corresponds to the finding of a cure for each patient's pathology and therefore it is possible to estimate how much time will pass before this happens.

According to historical records of medicine and thanks to new statistical tools we can predict and assure that it is inevitable and only a matter of time to find the cure in relatively short times for most diseases.

The technology available in each time period varied and improved, as a result of which the time to find cures for certain diseases was also drastically reduced. For example, for spinal muscular atrophy (SMA), which is a disease affecting muscles and movement first described in 1891 [28], gene therapy with the drug Zolgensma was implemented in 2019 [29]. In cases like this, finding a partial or total treatment took more than 100 years, but the time to cure diseases is not linear and is tied to technological and medical advances. Sometimes the cure is partial and sometimes it is total, so today finding estimated cure times for the diseases being fought cannot be calculated by averaging the number of years it takes to find each cure, but requires a specialized statistical study.

The life expectancy for cases of deaths related to diabetes and HIV diseases in the cities of Denver and Colorado, in the United States between 1990 and 2015 inclusive, was statistically calculated with real information. It is estimated for these cases a percentage of success in finding a cure, which varies based on time. An 80% success rate of finding a cure within the next 23 years is estimated, as

well as a 50% chance of finding a cure within the next 10 years, which are very likely statements for researchers with expertise in these diseases [30].

Based on these statistics and thanks to technological advances, it can be concluded that the cryopreservation of the patient will not be for an indefinite period of time, nor for hundreds or thousands of years.

It is not possible to calculate an exact time in which the cure for a certain disease will be found, since the times are estimated by statistics. Knowing this, the numbers give an approximate picture as far as realistic times are concerned for the wait prior to the de-cryogenization of the body. For this reason and based on the above statistics we estimate a time of less than 100 years of preservation of the patient to estimate the temperature at which the body should be cryopreserved, although it is advisable to calculate the times statistically for each patient and their pathology prior to cryopreservation of the body and also take into account the development of resuscitation technology. In this way the approximate estimated time will be known as well as the eventual temperature variations that could be made, even once cryopreservation has begun.

### ***Not exceeding -100°C is a safe temperature for the procedure***

Based on these statistics, the estimated preservation times of the cryopreserved body is despicable compared to the enzymatic reaction at -120°C which takes 34253 years to complete [See Table 2]. On the other hand, without taking into account the vitrification factor and analyzing the values of the same table, it is possible to take as feasible an even higher value as far as temperature is concerned for the preservation of the body, this is -100°C and even -95°C. These are values that does not contemplate vitrification and therefore require an improvement in the technique of using cryoprotectants to be able to be carried out. It is contemplated as a temperature at which the body would also be in an ametabolic state suitable for preservation if the formation of ice could be avoided.

Based on these estimates we performed the confirmatory calculations for table 1. And then we designed Table 2 with additional data using the Arrhenius formula, which is confirmatory to Table 1.

At the proposed temperature of -125°C it would take the ischemic process that delays 6 minutes at 37°C a time of 146858 years to take place (See Table 2), which is more than sufficient time and it prevents the possibility of fractures.

### ***Inhibitors allow the use of higher temperatures by increasing enzymatic reaction times***

Although LDH was used as a representative of (energy) metabolism and is useful for this purpose, it is recommended to consider the activation energy of caspase enzymes as the main cause of cell death, which can be induced intrinsically or extrinsically, the most important being caspases 8 and 9 that trigger the following cell death reactions [31]. In turn, these enzymes can be inhibited by introducing caspase inhibitors [32], which would increase the reaction time compared to not using inhibitors and thus result in longer reaction times at higher temperatures. Incorporating apoptosis and necrosis inhibitors such as zVAD-fmk, p38 MAPK inhibitor, ROCK inhibitor, etc. which inhibit caspases, proteases and kinases thus preserving the integrity of tissues and cells [13]. For this reason it is important to calculate the rates of initiator caspases rather than the rates of executioner caspases, since initiators are the ones that trigger the process of cell death and therefore it is important to know the energy required for their direct and indirect activation.

### ***Reducing the waiting times & protecting the brain parenchyma***

With respect to ischemia, although there is ischemia that is considered “warm” at the time of cardiac arrest, the greatest damage is that of “cold ischemia” that occurs when cryoprotectants are not applied in time. This second damage is greater and therefore it is essential to reduce the waiting times from the declaration of clinical death [8].

By reducing the temperature at the indicated times, a state of hypothermia is produced, which is optimal for protecting the blood-brain barrier and therefore the brain parenchyma [17].

This is why it is so important to promote laws that allow the procedure to be performed with controlled ischemia even prior to the moment of declaration of clinical death.

While there will be some damage under suboptimal conditions, most structures can be preserved [9] and by stem cell transplantation the tolerable ischemia time can be increased [10].

Although the amount of cryoprotectants to be used in the vitrification process should be evaluated to avoid high toxicity [11] as well as to determine which organelles are damaged by it in order to avoid it [12], the fundamental factor is to reduce metabolism as soon as possible by applying temperature decrease in time to reduce enzymatic reaction times and apoptosis.

With respect to brain structure, the vitrification mixture M22 is found to be successful in preserving structures in rabbits without ice crystal formation [24].

The results in other animals in which ischemia was produced and the time rate of the enzymatic reactions was reduced are more than satisfactory at temperatures of 0°C 10°C [9, 18-22]. It is for this and other reasons detailed previously that the use of temperatures higher than -196°C is proposed, the suggested temperature being -125°C. If the factors mentioned before are adjusted then -100°C is the temperature proposed.

### **Public policies**

The implementation of laws that allow for the early implementation of metabolic reduction treatment as well as anti-apoptotic treatment is fundamental for an optimal and successful outcome. For this, public policies must be implemented to ensure the understanding of the new concepts applied in this field, to move from what is considered “clinical death” to “ametabolic coma” [4].

The patient is not dead, he is simply in an “ametabolic coma” and must therefore be protected by the state. He is a citizen in his condition of a patient who requires immediate medical attention and not providing it to him goes against the guidelines of the World Health Organization, that states in its 1946 constitution:

- “...the highest attainable standard of health as a fundamental right of every human being.”
- “Acknowledging health as a human right recognizes a legal obligation on states to ensure access to timely, acceptable, and affordable health care.”
- “A state’s obligation to support the right to health - including through the allocation of maximum available resources to progressively realize this goal - is reviewed through various international human rights mechanisms.”[33].

In the words of Dr Tedros Adhanom Ghebreyesus, Director-General, World Health Organization, who states:

“No one should get sick and die just because they are poor, or because they cannot access the health services they need” [34].

It is proposed in this work that “clinical death”, henceforth considered as “ametabolic coma” is a reversible process and should be communicated in this way for the public knowledge. It can thus be enforced as a right, and it is for this reason that the work refers to cryopreserved individuals as “patients”.

Under current law, a person declared clinically dead loses his or her rights and becomes a thing. Once reanimated they fall outside of a legal framework because they are no longer the same person in legal terms. This is not correct and therefore a pertinent adjustment is required regarding the legality of the rights of the person receiving treatment. In other words, a legal redefinition is required [15].

Within the primary health care referred to in the WHO, which involves all social sectors, there should be community participation [35], including health education. It also states that efforts should be made to minimize the suffering caused by loss of health and to facilitate the adaptation of patients to incurable problems [36, 37], which is clinical death today considered as incurable. As we have

previously observed, this concept of being incurable only applies to the current technology available and not further beyond in time.

A last but not least consideration is the adoption of interest in this technology, which is not only beneficial for the rights of the citizens, but from the economic point of view of a country is a synonym of progress [25] and therefore helps to position a country for its scientific and technical potential [26].

## Conclusions & Future Work

### *Key findings and Conclusions*

- The cryopreservation procedure should be taken as the only viable current alternative to protect and preserve life, since it stops or significantly reduces the biological time, thus allowing the finding of a cure for the disease that causes death.
- The estimated time the patient will be cryopreserved must be calculated statistically and on an individualized basis.
- Decisions must be made as to the exact temperature of the procedure based on the previous statistics of each patient, in order to avoid possible fractures of structures given at a lower temperature.
- It is necessary to use the appropriate dose of cryoprotectants taking into account the requirement of each organ and tissue as well as their toxicity level. It is suggested to continue the use of Propofol 2,6-diisopropylphenol, heparin, streptokinase, THAM buffer, epinephrine and vasopressin and with the apoptosis and necrosis inhibitors zVAD-fmk, p38 MAPK inhibitor, ROCK inhibitor.
- To use the previously mentioned compounds to prevent or decrease proapoptotic processes and to evaluate new compounds if necessary. Calculate how the amount of these compounds increases the enzymatic reaction time, especially in terms of cell death, in order to evaluate the possibility of using higher temperatures, meaning above  $-125^{\circ}\text{C}$ .
- It is necessary to avoid temperatures close to  $-196^{\circ}\text{C}$  because of the possibility of fracture due to thermal stresses.
- Preserving patients at a temperature of  $-125^{\circ}\text{C}$  in a vitrification state that avoids crystallization due to ice formation is a prudent and very efficient practice, this is a safer method.
- The statistical times of obtaining the cure of the disease, previously calculated, should be considered in order to evaluate whether it is possible to use a higher temperature, which increases the possibility of subsequent reanimation of the patient.
- Based on the response of the survey, it is necessary to promote public education policies to improve cryonics education in public education.
- It is imperative to promote and encourage the legal redefinition under the new understanding of the concept of "ametabolic coma". Consequently, it is necessary to raise a law that guarantees the efficient performance of cryonics procedures and the readiness of the patient's body to begin treatment without delay.
- Cryonics is the only tool capable of dealing with "clinical death", giving the patient in ametabolic coma a temporary bridge to the future and the possibility of accessing the technology that can reanimate him and cure his disease.

### *Final considerations & Future work*

It is not necessary to reach  $-196^{\circ}\text{C}$  for an optimal procedure, a higher temperature can be used and it is dependent on the addition of apoptosis inhibitors. Inhibitors reduce the enzymatic rate and therefore the temperature can be higher, thereby altering the duration of enzymatic reactions at a given temperature.

Apoptosis inhibitors can be added when the patient is not yet declared clinically dead. In this case, the inhibitors are administered intravenously and are available at moment zero of ischemia. Inhibitors administered intravenously prior to the declaration of clinical death do not pose an immediate risk to the patient's life, but rather a benefit. At the time of declaration of clinical death, when the time comes to perfuse with the cryoprotectant, part of the apoptosis will have already been prevented. Since the cryoprotectant also contains inhibitors, they are present at all times, from before the onset of ischemia, thus increasing the enzymatic reaction times that lead to cell death.



### **Future work**

1. To use temperatures between -95°C and -125°C to avoid risks of fracture associated with lower temperatures. Avoid using standard temperatures; instead, calculate them based on enzymatic reaction times and each patient's pathology.
2. To implement the use of inhibitors prior to clinical death, in order to use higher temperatures to preserve the patient's body.

Cryogenics is an absolutely necessary tool that provides us with more time working with the same patient. It provides a temporary pause in the decomposition of the body and allows us to reanimate it, and the subsequent treatment of its pathology. An achievement impossible without its utilization.

While we advance in creating new technologies and addressing current challenges, cryonics allows the possibility of treating the patient without a time limit, it provides access to a temporary pause in the treatment, which otherwise would not be possible.

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