Alcor
A Non-Profit Organization

# Cryonics

April 2015 • Volume 36:4

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Alcor Life Extension Foundation
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# CRYONICS

*Cover Photo-Image Credit: Stephen Valentine, Architect*

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**9 The Stasis Foundation**
The Stasis Foundation is located in Comfort, Texas, and is part of an ambitious plan to build the most advanced patient care and research facility in the history of cryonics. The Stasis Foundation has conducted detailed studies into the next generation of patient storage options and is pursuing a number of other relevant research projects.

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21st Century Medicine is the largest cryobiology company in the world and engages in research to perfect the cryopreservation of complex mammalian organs. Their pioneering research in hypothermic organ preservation, low-toxicity cryoprotectants, brain vitrification, and whole body cryopreservation is of profound importance to cryonics and the prospect of human suspended animation.

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CRYONICS

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# 2015 Annual Giving Program

Alcor provides a wide array of services for you the member, and the general public. We inform and educate, we protect and preserve, and we strive to remain at the forefront of cryonics technology.

Since its founding, Alcor has relied on member support to maintain its mission and attract new members. Your support, regardless of size, can provide a better future for all cryonicists. **Please act now.**

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Donations may be made via the Donations button on the Alcor website or by contacting Alcor's Finance Director, Bonnie Magee, at bonnie@alcor.org. Your donation may be made as a lump sum or divided into easy monthly payments. ■

# The James Bedford Society

Gifts have played a fundamental role in the cryonics movement since its earliest days. Dr. James Bedford, a man whose extraordinary vision led him to become the first person to be cryopreserved, and the first to make a bequest to a cryonics organization, exemplified the determination of the early pioneers of cryonics. We invite you to follow in his footsteps, and join the James Bedford Society.

The James Bedford Society recognizes those who make a bequest of any size to the Alcor Life Extension Foundation. If you have already provided a gift for Alcor in your estate, please send a copy of your relevant documents to Alcor's Finance Director, Bonnie Magee.

If you'd like to learn more about setting up a bequest, send an email to bonnie@alcor.org or call 480-905-1906 x114 to discuss your gift. ■

# EDITORIAL

By Aschwin de Wolf

I sometimes take for granted that cryonics members are aware of ongoing research projects aimed at protecting against ischemia, improving urgent response capability, and perfecting whole brain cryopreservation.

Perfection of whole brain preservation would occur if we could induce cryogenic temperatures in lab animal brains, rewarm the vitrified brains, and have them regain full function.

At cryonics conferences, scientists working at the four full time labs dedicated to improving cryopreservation technologies often make presentations about their recent work. Up until now, however, no one amalgamated all of the various research projects into one descriptive publication.

This issue of *Cryonics* magazine is dedicated to enlightening Alcor supporters about the most aggressive research projects in human history whose goal is to improve the quality of cryopreservation in a way that will enhance odds of successful resuscitation.

None of the research that will be described in this issue is conducted by Alcor, but Alcor members derive full benefits from it because the four independent labs conducting this research routinely disseminate their findings to Alcor personnel. Alcor conducts its own in-house research and development to improve its cryopreservation capabilities.

The most important step cryonics members can take now is to extend their healthy life spans in order to avail themselves of these rapidly evolving technologies.

One way of doing this is to become a member of the **Life Extension Foundation** of Ft. Lauderdale, Florida (lef.org). This group has a track record dating back to **1980** of being light years ahead of the medical establishment in identifying methods to protect against disease, slow certain aging processes, and provide treatment protocols that statistically improve survival of those stricken with serious illnesses.

*"This issue of* Cryonics *magazine is dedicated to enlightening Alcor supporters about the most aggressive research projects in human history whose goal is to improve the quality of cryopreservation in a way that will enhance odds of successful revival."*

What motivates so many Alcor members to support the Life

*"Perhaps the strongest reason to patronize Life Extension is the fact that it is operated by long-time cryonics members who don't want themselves, or any of their members to ever die."*

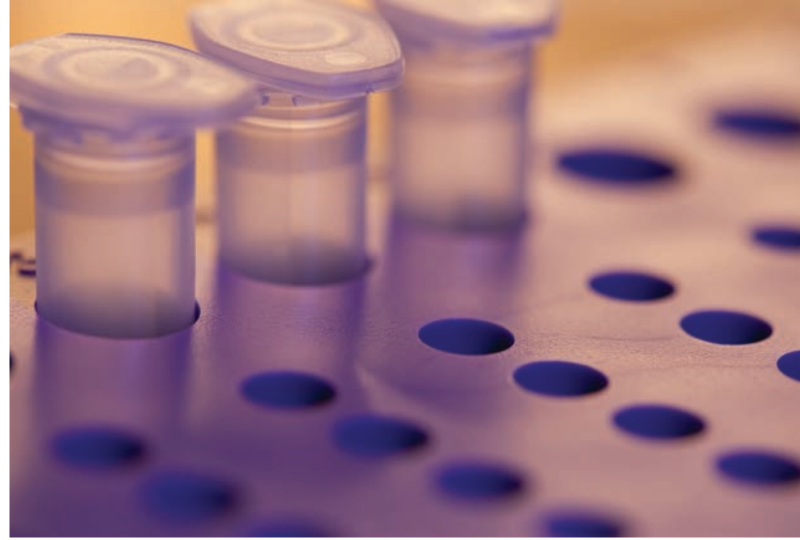
Extension Foundation is that this non-profit group fully funds all four of these research labs and they have set up several organizations and trusts dedicated to funding reanimation research in perpetuity. The total value of these various reanimation organizations is today in the hundreds of millions of dollars.

The monies to fund these four research labs plus the reanimation organizations come solely from membership dues ($75/year) and royalties received on sales of advanced dietary supplements, comprehensive blood testing, and small donations.

Long term cryonics members support Life Extension Foundation's efforts by patronizing companies that pay the Foundation a substantial royalty on every product or service. Life Extension Foundation then uses these funds to support these research labs.

I know every vitamin company likes to pretend they're the best, but Life Extension can prove it with an unparalleled track record of innovation. Perhaps the strongest reason to patronize Life Extension is the fact that it is operated by long-time cryonics members who don't want themselves, or any of their members, to ever die. No other company on earth can make this claim.

After you read the brief articles in this issue about the research programs funded by the Life Extension Foundation, I hope you'll consider joining this group and using their novel technologies to extend your life years, while simultaneously funding the many research projects they directly support. ■



**By Catherine Baldwin**

I have the coolest job, ever. Literally. I work for Suspended Animation, Inc. (aka "SA") whose mission is, in the simplest terms, all about cooling. Funded by the Life Extension Foundation and contracted by Alcor, SA provides nationwide standby and stabilization services for Alcor members. And, protecting cells and tissues by rapidly cooling the body is SA's primary aim for stabilizing Alcor members who are far from Alcor at the time of legal death.

There are various methods for bringing the body's temperature down to near 0 degrees Celsius to protect the brain and other tissues from damage during transport to Alcor. The methods haven't changed all that much over the years but there have been improvements, particularly in how they are delivered to cryonics patients, and there are new techniques on the horizon. These are the areas of SA's research and development. We spend a lot of time trying to figure out how to cool patients faster, more safely and more effectively.

Since opening in 2002, SA has developed new equipment and adapted state-of-the-art medical equipment and techniques for cryonics patient stabilization. SA also created a whole new model for training and contracting medical professionals like thoracic surgeons and perfusionists to perform standbys and stabilizations. These medical professionals are trained by SA to deal with the special problems of treating cryonics patients. They keep sharp by working regularly on medical patients in hospitals and other medical facilities, but are available on quick notice to travel to standbys of cryonics patients, where they use their considerable training and experience to provide quality care to these patients.

Nearly every year brings us new and improved technologies in the form of smaller computers and batteries, fast fabrication with lighter and stronger materials, and new laboratory and medical devices and techniques. With these developments come opportunities to incorporate some of these improvements into our existing protocols and equipment.

For example, there are few measures of quality and effectiveness of treatment and procedures performed on cryonics patients. Traditional laboratory methods generally involve biopsies and destructive tissue testing. There may now be some alternatives to this type of destructive testing.

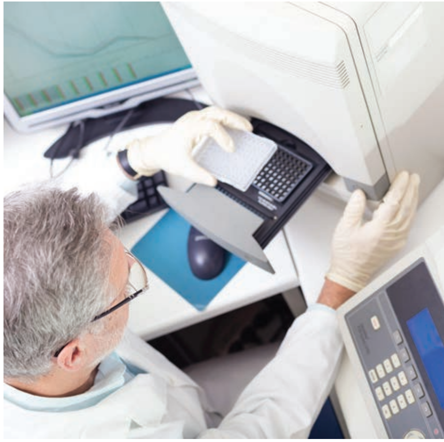
Newer functional genomic studies and gene expression analysis technologies have allowed scientists to identify many of the genes and signaling molecules expressed during cell stress, cell injury and cell death. There are genes involved in activating pathways that tell the cell to die and others involved in blocking these pathways. While these genes are expressed in the cells that make up the brain and other tissues, the molecules also wind up in the blood and cerebrospinal fluid (CSF).

SA is experimenting with using biomarker and gene expression analysis of the blood and CSF to try to assess the effectiveness of stabilization treatments, both generally and for individual cryonics patients.

During animal and human stabilization procedures, serial blood and fluid samples are collected. The samples are processed at SA's laboratory into commercially available assay plates and analyzed in a real-time quantitative polymerase chain reaction machine (Applied Biosystems 7500 Fast). This instrument is like a copy machine for genetic material present in our samples in tiny, and unknown quantities. Each plate contains 12 controls and 84 assays of the key genes involved in regulating cell stress and cell death responses. Over time, by examining and quantifying which genes appearing in our samples are consistently upregulated and downregulated during our procedures, we hope to better assess the quality and effectiveness of stabilization treatments. This may also allow us to test the effectiveness of proposed new treatments.

*Suspended Animation laboratory in California*

SA's present and future cooling devices are also getting an overhaul and benefitting from new research and technologies. Using advanced design modeling, computer simulations and 3-D printing, SA is developing high-powered cooling devices that offer real advantages over previous systems.



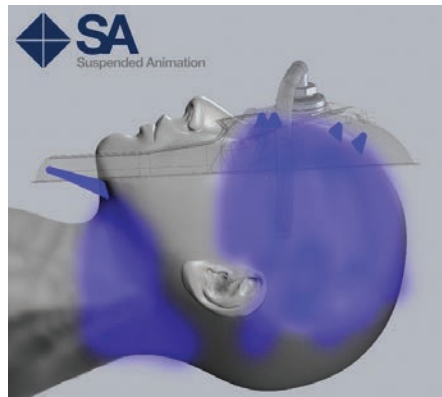
*Gene expression assay using the Applied Biosystems 7500 Fast*

Cooling a cryonics patient on the outside, particularly their head and neck, is the most basic step in the initial stabilization protocol. Simply packing a person in ice offers some cooling, but cooling power increases by flowing iced water over the skin and the more the better. The ice-cold water comes in contact with more surface area and can remove more heat. It's a bit like the difference between placing a can or bottle in a bucket of unmelted ice versus immersing it in a cooler of ice and water (which cools it faster). Other procedures being administered make it impractical to immerse a cryonics patient so, over the years, a variety of systems of pipes and tubes and large electric pumps have evolved to recirculate ice water within a shallow portable ice bath for the patient. Some worked better than others. We felt it would be worth the effort to figure out how to maximize flow over the head and neck to eke out some additional cooling benefits while eliminating some weight and safety issues associated with other systems.

SA did initial research and testing to identify the best off-the-shelf ultra-light submersible pump that was inexpensive enough to be disposed after use and still delivered high flow when converted to run from a battery instead of being plugged into a wall outlet. We found a 12-volt pump that weighs about a pound and pumps up to 1100 gallons per hour.

High flow pumps running for hours require significant power. Fortunately, high capacity lithium ion batteries continue to get smaller and more affordable to support computers and cell phones. We were able to find a small laptop battery pack with adjustable voltage that could power the high flow pump for almost a whole day at its maximum output.

Next, SA prototyped some mask designs using fiberglass and tubing, testing each for optimized flow. Each of these designs took several days to cut, form and cure—a very slow process. To speed things up, SA's staff designer recruited a group of industrial design students at the Art Institute of Pittsburgh who were eager to apply their skills and create a unique final class project. Using the latest computer aided design (CAD) and 3-D modeling software, SA and the students were able to experiment with more complex, multi-layered designs. A 3-D printer could produce a mask prototype from a design in a few hours. One mask design quickly emerged from testing as by far the best.




*Suspended Animation's cooling mask*

SA now has this mask fabricated overseas, blow molding it from just a few ounces of lightweight, disposable plastic. The mask delivers almost 11 gallons of ice water per minute directly to the critical areas of the head and neck of the patient without risk of spraying team members nearby. The power supply is small and rugged and can quickly charge phones, cameras and other devices in the stabilization kit as well as run the high flow pump for hours.

While fast-flowing ice water over the outside of the body can be performed almost immediately and offers some cooling, it's fairly slow with different parts cooling at different rates, around a degree or two of Celsius per hour. Slower cooling means more damage to tissues. So, how can we cool faster?

In hospitals treating victims of cardiac arrest or trauma, fast cooling is accomplished by surgically accessing large blood vessels in the heart, connecting them to a machine that circulates blood and cools it (called hypothermic perfusion). Cooled blood circulates to the brain and other tissues, cooling them. This technique can cool the body by a couple of degrees in a few minutes or to near 5 Celsius in 30 minutes to an hour.

SA acquired the same machines and modified them to be air-transportable. We then recruited on-call professional surgeons to perform the surgical procedure and perfusionists to run the machines to perfuse and cool cryonics patients. This is part of our standard protocol for stabilizing cryonics patients prior to transport to Alcor. A caveat is, whether for a trauma victim or a cryonics patient, performing this surgical fast cooling procedure requires a number of highly trained people, specialized equipment and a suitable facility. This can take time when what we need is *immediate, fast* cooling. Researchers at Critical Care Research, Inc. (CCR) and SA are developing a device that may solve this problem and act as a kind of immediate, fast cooling "bridge" until the surgical procedure can be performed.

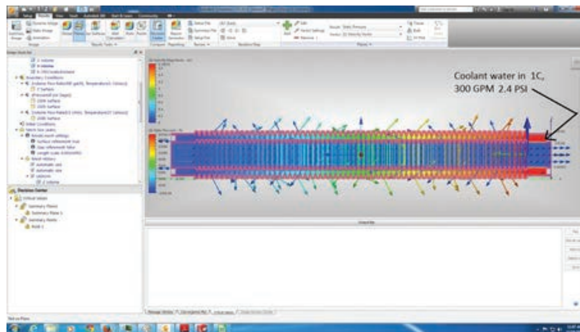
Like the surgical fast cooling procedure, the new device cools the blood and circulating this cooled blood cools the brain and other tissues. However, the new device does not require any surgery to access blood vessels. It uses the already open passages through the mouth and throat to access the millions of tiny blood vessels in the tissue of the lungs.

All of the body's blood passes through the lungs to pick up oxygen that we inhale and release carbon dioxide waste we then exhale. The lungs are ideally suited for this gas exchange because they contain a huge surface area that is dense with blood vessels at a thin interface. What researchers at Critical Care Research found was that the lungs could work very well for heat exchange as well as gas exchange.

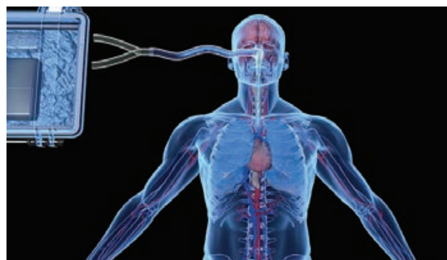
Similar to passing ice cold fluid over the outside of the body to remove heat, passing ice cold fluid over the inside surface of the lungs removes heat also, only much faster. Cycling cold fluid in and the warmed fluid out repeatedly cools the blood moving through the blood vessels in the lungs, which cools the patient. Connecting a patient to the device that pumps and cools the fluid requires only an endotracheal tube in the patient's airway to guide the fluid in and out of the lungs.

In 2013, CCR and SA were granted the patent for the "Portable Apparatus and Method for the Administration of Heat Exchange in the Lungs of a Mammal." In laboratory experiments, this "liquid ventilation" device for cooling cooled as fast as 1.5 degrees Celsius per minute in a live dog. Research by SA continues to further develop this device for use outside the lab.

At SA we have been taking advantage of the most advanced microelectronics and electromechanical systems to automate the manual functions of the machine. Using the same technology that allows computers and cell phones to grow smaller and more powerful, multi-layer circuit boards and low power chipsets have made the controls and interface of the new liquid ventilation machine lighter, simpler and able to integrate with other medical devices. SA engineers are researching new materials and new hardware configurations to maximize heat exchange with computational fluid dynamics (CFD) software and finite element analysis (FEA). Our goal is to make the device's components rugged, lightweight, inexpensive and largely disposable. ■



*Computational fluid dynamics software to analyze heat exchange efficiencies*


*Patient connected to the portable liquid ventilation device for cooling.*


# Become An Alcor Associate Member!

Supporters of Alcor who are not yet ready to make cryopreservation arrangements can become an Associate Member for $10/month (or $30/quarter or $120 annually). Associate Members are members of the Alcor Life Extension Foundation who have not made cryonics arrangements but financially support the organization. Associate Members will receive:

- ***Cryonics* magazine by mail**
- **Discounts on Alcor conferences**
- **Access to post in the Alcor Member Forums**
- **A dollar-for-dollar credit toward full membership sign-up fees for any dues paid for Associate Membership**

To become an Associate Member send a check or money order ($10/month or $30/quarter or $120 annually) to Alcor Life Extension Foundation, 7895 E. Acoma Dr., Suite 110, Scottsdale, Arizona 85260, or call Marji Klima at (480) 905-1906 ext. 101 with your credit card information.

Or you can pay online via PayPal using the following link: http://www.alcor.org/BecomeMember/associate.html (quarterly option is not available this way).

Associate Members can improve their chances of being cryopreserved in an emergency if they complete and provide us with a Declaration of Intent to be Cryopreserved (http://www.alcor.org/Library/html/declarationofintent.html). Financial provisions would still have to be made by you or someone acting for you, but the combination of Associate Membership and Declaration of Intent meets the informed consent requirement and makes it much more likely that we could move ahead in a critical situation.

# The Stasis Foundation Research

**By Stephen Valentine, RA (NCARB)**

## INTRODUCTION

Timeship, the primary project at the Stasis Foundation Research Park, will be a state-of-the-art next generation cryonics facility. Timeship will provide secure long-term cryostorage for the DNA of endangered species, organs for transplantation, and both neuropatients and whole-body patients for future reanimation. It will also house laboratories for advanced life extension research.

*Model of Timeship viewing the lower cryostorage area divided into twelve communities. The hierarchy of Neighborhoods and Communities allows for security and redundancy. The inner square is for $LN_2$ storage and cryogenic equipment.*

The site is approximately 800 acres in Comfort, Texas, less than one hour northwest of San Antonio. Plans are currently being drawn up for the first phase of a fabrication and testing laboratory to further develop an advanced intermediate temperature storage system referred to here as the Temperature Controlled Volume (TCV), a cryostorage container with Timeship Cold Volume features. Once we have a proven working prototype of this container, the next phase will be the development of the Cold Volumes to be used in Timeship.

While there have been decades of research in cryopreservation, there is also the issue of the contextual requirements for cryostorage. While most buildings are for well understood activities, just about everything that will take place at Timeship will be unique and technologically demanding, including determining the site of the building in terms of climate, safety, infrastructural and social issues. There will also be the need for architectural and mechanical systems of high reliability requiring minimal maintenance for extended time periods. These requirements necessitate that Timeship be not just an architectural and construction project, but also a major ongoing research project. Years of research have been committed to the project, often involving the world's leading engineers.

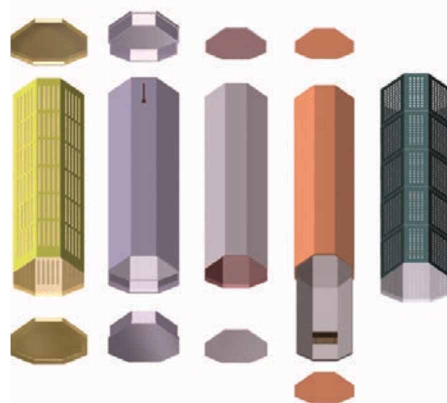
Timeship has been designed to be a "building of immortality" both in its program and its construction. The products of this research to date are contained in a massive multi-volume Program Report and summarized in the book, *Timeship: The Architecture of Immortality.*

In this article we will focus on the cryostorage systems at Timeship, very briefly list some of the Foundation's studies of the literature of cryopreservation and life extension, and briefly describe the library being established by the Stasis Foundation.

There are four components to the Stasis cryostorage systems: Pods, Transport Containers, Temperature Control Volumes (TCVs), and Cold Volumes. Until Cold Volumes at Timeship are operational, cryonics patients would have to be shipped in Transport Containers (which are not addressed in this article) to a secure location where they would be placed in TCVs. Once Timeship is operational, the patients will be removed from the TCVs and placed in Cold Volumes where they will remain until reanimation. One of the features of this system is that once a patient is placed in his or her Pod, he or she will not be removed from that Pod until reanimation.

## PATIENT & ORGAN PODS

There are two types of Pods, "whole-body" Pods and "organ" Pods that are used for both organs and neuropatients. Pods must provide both impact and thermal protection for the fragile vitrified patients. Toward this objective, a complex design was developed with multiple layers, each layer having a protective or thermal role in bringing the patient safely to eventual reanimation. From the outside-in, the layers of the whole-body Pod are as follows: first is a protective enclosure tube with removable top and bottom. Within this tube goes a stainless steel vacuum-insulated volume and an aluminum heat-conductive liner to assure that the patient is kept at an even temperature. The patient capsule is placed inside a liner, and inside that the patient cradle is inserted.



*The multiple components of the Full Patient Pod*

The Pod is the basic module of Timeship, and the entire building is designed outward from the Pod. The Pod design must accommodate the shape of the patient on the inside, and the way Pods are

packed (fit together) on the outside. The packing geometry of the Pods is crucial to the efficient cryostorage of a large number of patients.

## TCV (TEMPERATURE COLD VOLUME)

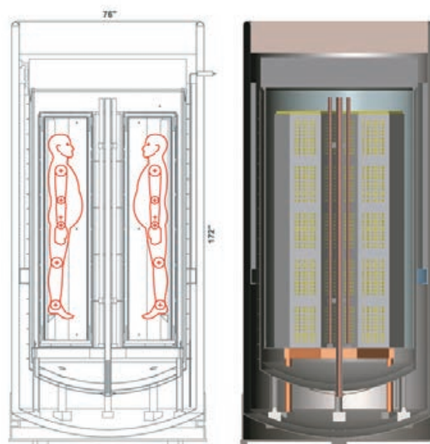
For the most part, since its inception cryonics has depended on storing patients in Dewars—vacuum double-walled vessels like giant Thermos bottles—filled with liquid nitrogen ($LN_2$) that provide a reliable constant temperature of -196°C. However, the advancement of methods of cryostorage using vitrification requires a higher temperature of approximately -130°C. In addition, future developments in vitrification may require different temperatures for different patients, and current techniques may require varying the temperature during cooling to produce annealing to minimize fracturing. While commercially available refrigeration may be able to meet these requirements, such refrigeration involves compressors, which are not reliable over the long time periods required for cryostorage. The Stasis Foundation and its engineers have done extensive investigations to address these issues.

The solution is the TCV, or Temperature Control Volume, which was developed by cryogenics designer Michael Iarocci, physicist Dr. Brian Wowk, and Stephen Valentine, architect of Timeship. The TCV allows storage at temperatures compatible with vitrification, and will be used as stand-alone units containing the Pods described above until Timeship is operational, at which time the Pods will be removed from the TCVs and placed in Timeship's Cold Volumes.

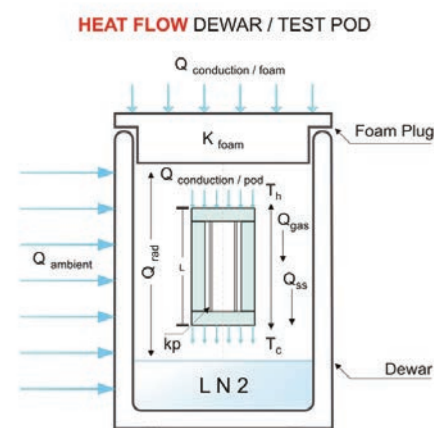
The TCV must be physically secure, provide cooling and precise and variable temperatures, and have sufficient insulation, structural integrity, and shock resistance to permit some limited transport.

In the TCV, a vertical open mouth Dewar houses a second open mouth Dewar, all constructed of stainless steel. Both vessels have vacuum insulation and superinsulation to minimize heat transfer, and generally the vessel wall vacuum spaces are common to facilitate re-evacuation from the outside.

Heat conductors are positioned at the bottom of the TCV so that they can be immersed in or removed from an $LN_2$ bath. Adjusting these conductors via control cables changes the thermal resistance between the TCV bottom surface and $LN_2$ pool, and changes the heat transfer rate to the $LN_2$, thereby allowing control of the temperature of the TCV. Small electric resistance heaters are located within the pod's surface to "trim" and more finely control the temperature as required. The heat flow of the TCV is modeled analytically using the electrical resistance analogy approach.




*Section Drawing: Proposed TCV Version [4] Dewar with Full Patient Pods.*



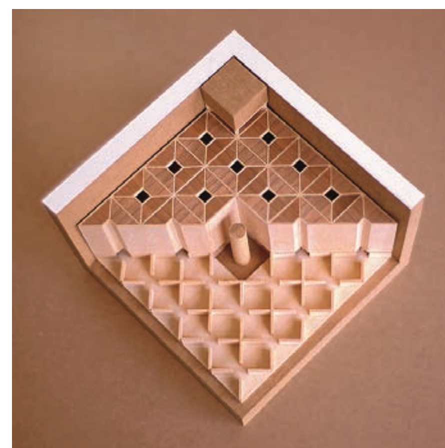

*Schematic for modeling the heat flow in a Pod with a highly conductive liner contained within a highly insulated Dewar.*

## COLD VOLUME STORAGE

The objective of Cold Volume storage rooms at Timeship is to provide a large-scale economical method for long-term storage for human organs, neuropatients, and whole-body human patients in a secure cryogenic environment with particular attention to equipment simplicity and redundancy to optimize reliability and safety. This approach was first proposed by Dr. Steven B. Harris in 1986.

Each Cold Volume will contain many Pods. New patients in their Pods will be brought into the upper level of the Cold Volumes through airlocks to protect against cold escaping and moisture getting in. Working in this upper level, Timeship staff will lower the Pod through access hatches into the appropriate slot in a manner that minimizes perturbations to the temperature of the Pods and facilitates safety for staff involved in transfers.

Individual Cold Volumes are referred to as "Neighborhoods," which are 7m × 7m × 5m high and are configured in a 3 × 3 matrix of nine each to form "Communities." Assuming whole-body storage, a Neighborhood could hold 100 patients, a Community could hold 900 patients, and the entire Timeship complex could hold 10,800 patients. If storage included some percentage of neuropatients, the numbers would be higher.



*View looking into a neighborhood with about half of the pods in place.*

Surrounding each Community in corridor spaces are local $LN_2$ supply Dewars for the cooling of each Neighborhood.

The corridors will also provide the space for the process systems that will maintain the required temperature for each Cold Volume.

Evaporation of $LN_2$ provides all of the refrigeration requirements for the Cold Volumes through the heat absorption property known as heat of vaporization. The $LN_2$ is injected upstream of the cold circulating pump into a diffuser section, passes through additional diffusers at the pump outlet, then passes heaters to achieve precise temperature control before entering the cold volume. The nitrogen gas is then circulated by fans compatible with a cryogenic environment. The volumetric flow is expected to be minimized so cold pump work is minimized. With flow selected and the number of pods identified, the total volumetric flow can be determined. The quantity with consideration for hydraulic losses in the ducting, Pods, heats exchangers, etc., will enable the selection of the cold circulating pumps. With pumps and associated hardware selected for circulation of nitrogen gas, the needed quantities of cooling $LN_2$ injection can be defined. The Cold Volumes are held at a constant pressure slightly above atmospheric pressure to minimize the infiltration of air and moisture which would cause a buildup of frost.

The Cold Volumes are required to provide precise temporal and spatial temperature control so that temperatures in each Pod can vary over time in order to facilitate annealing during cooling. Therefore it is necessary that Pods be thermally isolated from each other so that heat does not interfere with annealing. During the annealing process normal convection cooling to the particular Pod is not disrupted, so only local Pod heating is required to maintain the increased temperature. After annealing the Pods slowly reach the base temperature over time. The base or nominal Cold Volume temperature is approximately -130°C. The temporal and spatial temperature control requirements are +/- 2.5°C and +/- 0.5°C respectively. The cooling scheme proposed will allow changes in the base temperature as required.

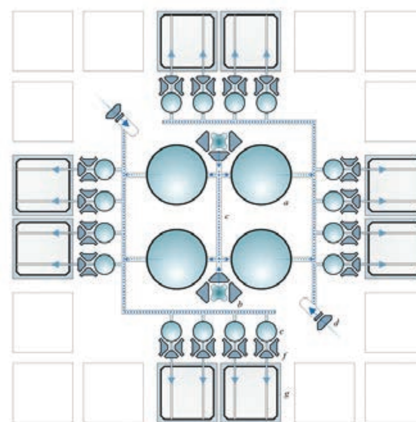
The areas in which the staff works are separated from the areas in which there is $LN_2$ by airlocks similar to those on a space station. Workers in areas with $LN_2$ or subject to $LN_2$ leaks will wear breathing apparatus for their protection and to prevent their exhalants from introducing moisture and other gases into the sealed environment.

Timeship must operate without interruption for up to hundreds of years and do so economically. The cost of operation is strongly influenced by the Cold Volume demands, type of circulation equipment selected, the geometry of the packing of the Pods, and the type of insulation used. Close packing of the pods in the Neighborhoods and Communities at Timeship is necessary for maximum energy efficiency. The final choice of packing geometry must consider the size of the pods in order to accommodate the greatest range of patients; the ratio of whole-body patients to neuropatients and organs; the interior volume to surface area ratio, which affects heat transfer; the packing properties of various geometries; and the configuration of the Neighborhoods and Communities in relation to the design of the building as a whole.

As mentioned above, the cooling equipment must be redundant at every level. In addition, each individual Cold Volume cooling apparatus shall interface with the adjacent Cold Volumes so as to add another layer of redundancy and flexibility. The $LN_2$ in Dewars on-site shall provide adequate reserves to cover anticipated $LN_2$ delivery interruptions and equipment failures as determined by a failure mode and effect analysis (FMEA). A careful FMEA early in the design phase of the project will assure elimination of fundamental design errors and can be a powerful tool in revealing any systemic weaknesses.

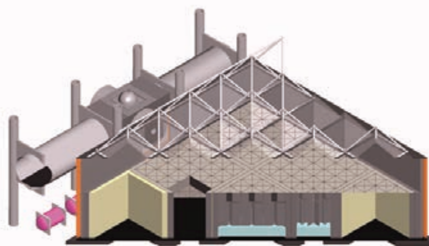
The storage area as a whole is isolated by thermal, structural, blast, and security barriers, as well as by the air locks mentioned above.

Once the Pods and TCV described above are fully developed and prototypes are built and tested, the internal Cold Volume space frame, transfer hatches, and transfer equipment can be initially designed.



*Timeship will keep a backup supply of $LN_2$ should deliveries be interrupted, and will also be able to make its own $LN_2$. This diagram shows the distribution of $LN_2$ within Timeship and the extensive redundancy of supply to each area.*

Then the next step in the development of cryogenic hardware will be the creation of a scaled version of the Cold Volume.



*A community of nine neighborhoods with access tube. The small pyramids at the top represent the structural truss system of the blast-resistant upper enclosure that provides a high degree of security. The open area under the upper enclosure is the patient transfer volume for maneuvering patients to be lowered into the suspension matrix grid.*

## INTERNSHIP RESEARCH

One of Timeship's mandates is to become a major repository of information regarding cryostorage, life extension, and related subjects. The material will be stored both online and on site in the Stasis Foundation Research Library.

The information is being gathered by teams of recent graduates working as interns-in-residence at the Stasis Foundation properties to survey the literature in various fields and compile reports. The interns are

generally from Texas universities and are coordinated by Stephen Valentine and the Stasis Foundation Director of Education and Research. The reports done to date, starting in late 2011, include:

- *Preserving Human Cells and Tissues: Techniques of Cryopreservation*
- *Cryovessels and the History of Liquid Nitrogen*
- *The Engram of Memory Encoded Through Synaptic Plasticity*
- *Memory, Consciousness, and Death*
- *Nanomedicine: Applications to Cryopreservation*
- *Organ Bioengineering*
- *Cryopreservation of Gametes and Embryos (A Historical Overview)*
- *Human Reproduction: Process of Collection of Gametes and Embryos for Cryopreservation (IVF)*

## RESEARCH LIBRARY

The Stasis Foundation established the Stasis Foundation Research Library (SFRL) to be a leading resource center for life extension research. SFRL is a unique organization with an ambitious mission: to provide resources to advance life extension research and to facilitate the innovation and discovery of new technologies that will transform lives and the future of humanity.

The collection will include, but will not be limited to, methods of cryopreservation, long-term organ storage, nanotechnology and brain science. The collection development process will be defined to include a plan for the future growth of the collection, a tool for delineating collecting priorities, and a guide for those charged with the responsibility of selecting materials.

It is a goal of the Foundation to provide free access to the Library's collection to the widest possible audience. This will be accomplished with on-site access along with interlibrary loan access through a system that will provide material directly from patrons worldwide.

With ongoing attention to detail the SFRL will become an indispensable resource for everyone from researchers to individuals new to the concepts of life extension. As SFRL emerges as a leading research institution, the Library will pioneer dynamic, user-focused methods for retrieval of information for continuing research in life extension.

## CONCLUSION

As mentioned at the beginning of this article, Timeship is as much a research project as an architectural project. The demands for up to hundreds of years of uninterrupted, precise conditions for Timeship's precious cargo are comparable only to the demands of the International Space Station. Timeship's design and engineering must meet this challenge. ■

## Reduce Your Alcor Dues With The CMS Waiver

Alcor members pay general dues to cover Alcor's operating expenses and also make annual contributions to the Comprehensive Member Standby fund pool to cover the costs of readiness and standby. Benefits of Comprehensive Member Standby include no out-of-pocket expense for standby services at the time of need, and up to $10,000 for relocation assistance to the Scottsdale, Arizona area.

Instead of paying $180 per year in CMS dues, Alcor also provides members the option to cover all CMS-associated costs through life insurance or pre-payment. Members who provide an additional $20,000 in minimum funding will no longer have to pay the $180 CMS (Comprehensive Member Standby fund) fee. This increase in minimums is permanent (for example, if in the future Alcor were to raise the cost of a neurocryopreservation to $90,000, the new minimum for neurocryopreservation members under this election would be $110,000). Once this election is made, the member cannot change back to the original minimums in the future.

To have the CMS fee waived, these are the minimums:

- **$220,000 Whole Body Cryopreservation ($115,000 to the Patient Care Trust, $60,000 for cryopreservation, $45,000 to the CMS Fund).**
- **$100,000 Neurocryopreservation ($25,000 to the Patient Care Trust, $30,000 for cryopreservation, $45,000 to the CMS Fund).**

If you have adequate funding and would like to take advantage of the CMS waiver, contact **Diane Cremeens at diane@alcor.org.**

# 21st Century Medicine

21st Century Medicine (21CM) is a biotechnology company specializing in complex system cryopreservation, and particularly the cryopreservation of whole mammalian organs. Research on organ cryopreservation is of great interest to those interested in cryonics, such as those who have signed up with Alcor. The full gamut of 21CM research is not described here, but recent research at 21CM that is particularly relevant to cryonics includes the following.

- As little as 1-2% ice formation in one part (the inner medulla) of a previously vitrified rabbit kidney during warming (devitrification) was damaging but was compatible with life support of the kidney after transplantation (providing the first proof of principle that whole organs can be vitrified and recovered in a viable state);

- using a new perfusion process and a variety of other new technologies, kidneys can now be perfused with a powerful vitrification solution (M22) well enough to become immune to devitrification at warming rates above 40ºC/min and yet maintain life support after this new perfusion process;

- using technology developed at 21CM, uniform electromagnetic warming of volumes the size of rabbit kidneys can be done at up to 160ºC/min without thermal runaway, and projections indicate that similar warming should be possible even on human sized organs, so that rabbit kidney technology should be scalable to the size of human organs, potentially allowing demonstration of large organ survival after vitrification;

- fracturing can be prevented in human organ sized solution samples even after cooling to liquid nitrogen temperature, and organs themselves should be even less liable to fracturing than these liquid samples;

- published 21CM data indicate that the formation of new ice crystals (ice nucleation) in M22 should be almost totally suppressed at temperatures of about -140ºC;

- published 21CM calculations indicate that temperatures of about -140ºC should permit safe storage of living systems (no biological deterioration) for extremely long periods of time (tens of thousands of years, or longer);

- the vast majority of vitrified organs of 70 kg (human-sized) pigs (including the kidney, heart, liver, and brain) can be reliably cooled to -140ºC without fracturing;

- using both refractometry and differential scanning calorimetry, 21CM has been able to predict non-invasively when rabbit kidneys prepared for vitrification are ready to be vitrified;

- using similar techniques, 21CM has been able to show that the limiting factor for kidney vitrification is small variations in cryoprotectant concentration from microregion to microregion of the renal inner medulla (see YouTube video of Dr. Fahy's symposium presentation at the 50th anniversary meeting of the Society for Cryobiology at https://www.youtube.com/watch?v=weeOjfQZtX0 );

- extending this observation, 21CM has shown that the same phenomenon of incomplete uniformity of cryoprotectant distribution applies to all 40 different tissue types tested in both rabbits and pigs;

- highly preliminary evidence suggests that new 21CM methods might be able to overcome or at least blunt this limiting factor for vitrification of the kidney and other organs, even when the organs are damaged by warm ischemia or prolonged cold storage (or even the combination of the two) prior to cryoprotectant perfusion;

- diagnostic methods for imaging and quantifying ice formation throughout large, mostly vitrified

3-dimensional objects are possible using physical sectioning below the glass transition temperature to expose structural details and allow sampling of tissue sub-regions for detailed investigation, including determination of how much damage is done by incomplete vitrification;

- amide toxicity can be neutralized in kidney, liver, and brain slices (which shows that 21CM technology is applicable to all of these systems, again to the potential commercial benefit of 21CM, but which also implies that the benefits of solutions like M22 might be applicable to whole brains);
- using sophisticated HPLC methods developed at 21CM, 21CM research has shown that all of the cryoprotective agents in M22 penetrate into multiple organs, including the brain, about equally rapidly, even though, in the brain, shrinkage is observed;
- brain (hippocampal) slices from both adult rats and adult rabbits can be vitrified and rewarmed without any losses of structural integrity, viability, or functionality (including responsiveness to electrical stimulation, action potential amplitude and conduction velocity, etc.); this is critical for 21CM's goal of marketing vitrified organ slices to pharmaceutical companies and might be able to provide a method for obtaining cellular resolution of the 2-deoxyglucose method for probing basic features of brain physiology, as discussed elsewhere (but it also provides the beginnings of a proof of principle for the possibility of whole brain cryopreservation);
- complete survival of vitrified-rewarmed brain slices implies that brain slices are not susceptible to chilling injury (which not only provides information critical for deciphering the mechanism(s) of chilling injury, but also provides evidence that reversible brain cryopreservation by vitrification is not precluded by chilling injury);
- brain viability can be maintained for 15 hours or more at 3ºC (which may help rescue trauma victims and soldiers, but may also protect brains during preparation for cryopreservation);
- 21CM protection methods allow brain histology and ultrastructure to be very well preserved after one hour of warm ischemia in both rabbits and pigs (which may help trauma and heart attack victims as well as soldiers survive longer periods of cardiac arrest, but also provides evidence consistent with the possibility that brains can be successfully cryopreserved even after significant preceding warm ischemia);
- brain pre-fixation prior to perfusion with M22 appears to preserve normal-looking brain ultrastructure in the cryoprotected state (which not only may allow 21CM to win the Brain Cryopreservation Prize but also provides an alternative for some who may prefer this method of cryopreservation); and
- recent close examination of whether 21CM might be able to win the Brain Cryopreservation Prize via ordinary (potentially viability-preserving) methods of M22 perfusion has indicated that, by employing very high-magnification imaging methods, extensive synapses and intact membranes are readily visible in the cryoprotected state after all, without any particular interventions (which is obviously good news for the possible feasibility of cryonics).

In addition to work done directly at 21CM, 21CM research has inspired collaborative and even independent research in other laboratories. The most important examples of this type of inspired extramural research include the following:

- 21CM collaborative studies have helped to identify new mechanisms of chilling injury and cryoprotectant toxicity, both in blood vessels specifically (which are found in all organs, including the brain; results are currently being written up for publication) and in systems of potential commercial value (particularly liver slices; results already published), and 21CM analysis of these studies has suggested for the first time what the overarching mechanisms of both chilling injury and cryoprotectant toxicity may be;
- very recent unpublished major breakthrough studies have identified multiple independent interventions that have already resulted in radical improvements in cell survival or function after M22 exposure in model systems; these interventions appear likely to be helpful when applied to rabbit kidneys in *in vivo* in studies that are now being planned.

In addition to moving ahead with rabbit kidney vitrification using new interventions to negate toxicity, 21CM is also currently hoping to extend its work on rabbit kidneys to porcine kidneys. With luck, porcine kidneys will be found to be less variable in their response to M22 than rabbit kidneys, and they and their hosts will be more tolerant of M22 perfusion and transplantation. If this works as hoped, not only will it show that organs can be reliably cryopreserved, but it will also show that human-sized organs can be reliably cryopreserved. Obviously, if this is achieved, cryonics will become more credible as a byproduct.

On another track, 21CM was approached a few years ago by NASA for advice about how to freeze dead astronauts in space. And 21CM has been involved in discussions with the X-Prize administrators about the prospects for cryopreserving whole mice. While 21CM scientists believe the possibility of cryopreserving a whole mammal and reviving it is remote at this time, even the late Harold Meryman, a

foundational figure in the science and history of cryobiology, and no friend to cryonics, acknowledged that, when all other problems had been solved, the ultimate goal of cryobiology would have to be human suspended animation. And Audrey U. Smith, another pillar of the development of cryobiology as a science, undertook very extensive experiments on the revival of frozen hamsters, with illuminating and widely published results.

Consequently, when 21CM was approached, several years ago, by a wealthy family interested in providing grant support for whole mammal cryopreservation, it was decided that the project deserved some consideration. Although the task of reversible mammalian cryopreservation might require 20 years or more to come to fruition, it is also true that it will never happen if no research is ever done to at least explore the boundary conditions that may exist with respect to this problem. The long-term problems of cryopreservation for space migration seemed to deserve some preliminary study. Plus, there was an additional consideration of some weight, which was simply that, as much as cryobiologists and cryonicists have debated one another and tried to convince each other of the errors of their ways, what has always been missing from the debate has been adequately relevant facts. While strong opinions have always abounded on both sides of this argument, the cryobiologists have not had sufficiently convincing hard data to dash the hopes of the cryonicists, and the cryonicists have been equally lacking in data sufficient to refute the entirely reasonable negative inferences of the cryobiologists. Finally, the prospect of obtaining simultaneous information about the equilibration rates of all organs in the body was an interesting side benefit of the idea of doing research on whole mammals. Therefore, although this was not a project 21CM would have ever undertaken on its own, the value of receiving supplemental funding to enable this "luxury" project to be undertaken was deemed to be persuasive.

The results of this "non-partisan" investigation so far have given both sides of the cryonics debate some points. Under good conditions, good brain preservation is obtainable with rabbits, pigs, and sheep, brains being perhaps the most easily vitrified organ in the entire body, with preservation of both histology and ultrastructure. After 1 hour of warm ischemia, adequate results can still be obtained in both rabbits and pigs as well (sheep not studied). Similarly, cold storage or cold perfusion for 24 hours adequately preserves brain structure with and without subsequent perfusion with M22. In addition, the mean percent of brain mass that became ice under conditions that maximized ice formation after M22 perfusion (i.e., with devitrification; averaged over 21 independent samples per brain) was about 0.13% after 24 hours of static storage and about 0.75% after 24 hours of continuous perfusion prior to M22 perfusion, which is perhaps acceptable in both cases.

However, 1 hour of warm ischemia plus 24 hours of cold storage gives results that depend on the techniques employed. Static storage, which is the current norm, resulted in significant thalamic damage, whereas continuous perfusion enabled remarkably good preservation of brain structure. In addition, ischemia plus 24 hours of continuous perfusion resulted in a mean maximized (by devitrification) mass percent ice in the brain of about 0.1%, whereas ischemia plus conventional static storage resulted in almost 4.5% w/w brain ice.

Thus, these experiments do not resolve the debate between the pro- and the anti-cryonics camps. They do, however, seem to shed considerable new light on this very under-researched subject. They also suggest that additional research is justified, and that improvements in cryonics methods might allow significant progress in the future. ■

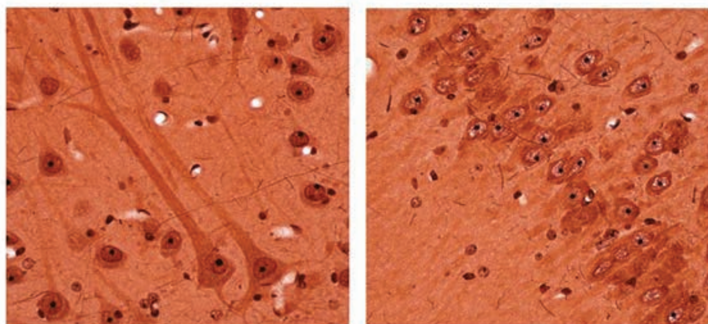


*Figure 1. Pig cerebral cortex on the left and hippocampus on the right after 1 hr of warm ischemia following 21CM mitigation. No cryoprotectant. General brain structure is preserved.*

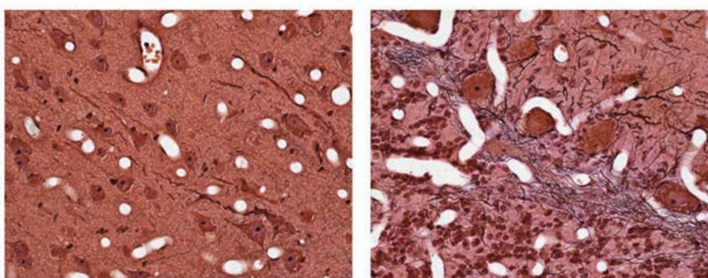


*Figure 2. Pig cerebral cortex on the left and (in higher magnification) cerebellum on the right after 24 hours of continuous cold perfusion followed by perfusion with 105% of full concentration M22. General brain structure is preserved. The white zones in both images are dilated capillaries, which is a typical osmotic effect.*

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**References**

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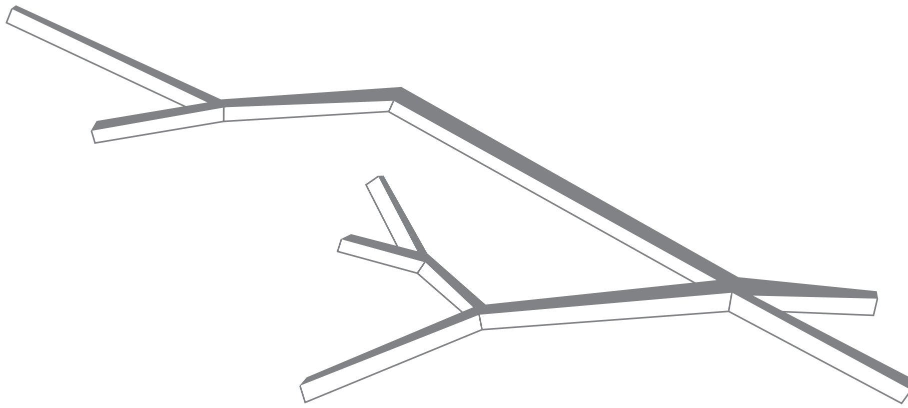
# advanced neural biosciences



**By Aschwin de Wolf and Chana Phaedra**

## Introduction

*Advanced Neural Biosciences* was founded in late 2008 by Aschwin de Wolf and Chana Phaedra as a result of a number of unexpected events favoring its creation; the departure of Yuri Pichigan at the Cryonics Institute, whose research equipment and supplies were made available to us; a generous private donation from Cryonics Institute member Alan Mole; free lab space made available in Salem, Oregon, by Jordan Sparks (the founder of Oregon Cryonics), and the strong support by Ben Best to engage in practical cryonics research.

Despite our innocuous name, our research was motivated from the beginning by the aim of validating and improving the science of human cryopreservation. From 2009 to 2014 our company conducted contract research in Salem, Oregon, initially on an uncompensated basis on weekends and later as a part-time endeavor. In late 2013, we relocated to a bigger lab space in Portland, Oregon, and secured sufficient funding to employ the two founders full time. In 2014, we expanded the company with a part-time employee and entered 2015 seeking to grow further.

Our animal model of choice is the rat. While the use of small animal models has often been challenged in aging research because of the limited relevance to larger mammals (including humans) because of genetic differences, we have found that in the case of ischemia and cryobiology research our results track findings in larger animal models and observations in humans well. In particular, our findings of the effects of cryoprotectants on the rat brain (blood brain barrier permeability, the effects of ischemia, etc.) appear to confirm what we are observing in human cryopreservation cases.

## Prior Research

We started our research using the red blood cell (RBC) to investigate the effects of different cryoprotectants and loading protocols on viability. While this model is too "crude" to distinguish subtle differences between cryoprotectants, or the effects of cryoprotectants on specific cell types such as endothelial cells or neurons, it provided us a basic method to understand the effects of cryoprotectants, as mono-agents or in combination. While we quickly moved on to more advanced research models, we occasionally have used the RBC model to look for acute toxicity issues in new cryoprotectants we are testing. We also used the model to shed light on a debate about the cryopreservation of James Bedford, in whose case it had been (erroneously) claimed that 100% pure concentrations of DMSO were used, which would have been, as we observed, highly destructive, at least to this type of cell.

Our first investigations into the effects of ischemia on the perfusability of the brain involved a transcardial perfusion model in which the brain was perfused with India Ink after various periods of cold or warm ischemia. Our most robust finding in that research was that rapid cooling after cardiac arrest protects the brain against the so-called "no reflow" phenomenon, especially if the blood is replaced by an organ preservation solution. This finding still stands today and validates contemporary cryonics procedures such as rapid ice bath cooling and remote blood substitution.

> *"If cardiac arrest is followed by rapid cooling and blood washout with MHP-2, ice-free cryopreservation of the brain is still feasible for at least 48 hours of cold "bloodless" ischemia."*

After researching the no-reflow phenomenon using ink perfusion we developed a model in which we investigated the effects of ischemia on cryoprotectant perfusion and vitrification. We have used this model extensively over the years to understand the effects of ischemia on cryopreservation of the brain, initially spurred and supported by the Cryonics Institute's desire to improve the quality of cryopreservation after periods of prolonged warm and cold ischemia and/or extended patient transport times. We described our findings in more detail in our 2011 *Long Life* magazine article "Human Cryopreservation Research at Advanced Neural Biosciences." (http://www.advancedneuralbio.com/publications/) In short, we validated prior research in the field and observed

that as the duration of warm and cold ischemia increases, the blood-brain barrier is progressively compromised, edema of the brain and the whole body increases, and delivery of the cryoprotectant to the brain is poorer, producing ice formation upon cryopreservation (despite the use of a vitrification agent). Most of our research efforts were aimed at identifying interventions and protocols to reverse this ischemia-induced perfusion impairment at the time of cryoprotective perfusion. While ice formation worsened when perfusion pressure was increased during cryoprotective perfusion in ischemic brains, we did observe benefits when we introduced the target concentration of the cryoprotectant without gradually increasing the concentration and we observed noticeable improvement when we increased the viscosity of the cryoprotectant. While the former approach is expected to improve protection against ice formation at the cost of (extreme) osmotic damage, the approach of increasing the viscosity of the cryoprotectant appears more appealing and we identified a number of potential carrier solutions and cryoprotectants that were successful in reversing ischemia-induced perfusion impairment and ice formation, especially in the case of cold ischemia. To our knowledge, this was the first time ischemia-induced perfusion impairment during cryoprotective perfusion was mitigated through modification of the carrier solution.

Our most successful results in a cold ischemia model were observed when we replaced the blood of the rat with an organ preservation solution. An important discovery was that the composition of the solution is important and the best results were seen when we replaced the blood with Alcor's MHP-2, a high-potassium, HEPES-buffered "extracellular" whole body organ preservation solution that includes the oncotic agent hydroxyethyl starch (HES). If cardiac arrest is followed by rapid cooling and blood washout with MHP-2, ice-free cryopreservation is still feasible for at least 48 hours of cold "bloodless" ischemia. This result was not observed with saline or other organ preservation solutions, with the exception of a "brain preservation solution" that was made available to us by 21st Century Medicine. Both solutions, however, were not effective in preventing (severe) whole body edema and weight gain during subsequent cryoprotectant perfusion, an issue that, to our knowledge, has not been successfully solved in the design of hypothermic organ preservation solutions. Preventing this type of vascular leakage (and breakdown of the blood brain barrier) may require a transition from "static" organ preservation solutions to intermittent perfusion of oxygenated solutions, continued perfusion en-route to a cryonics facility, or conducting cryoprotective perfusion in the field.

We also received a grant from LongeCity that allowed us to investigate whether blood substitution is still beneficial after various durations of normothermic (i.e., body temperature) ischemia—an important practical topic for cryonics organizations. This research was motivated by former Cryonics Institute President Ben Best's concern that attempting blood washout *after* warm ischemia could actually worsen subsequent cryoprotective perfusion by introducing free radical-mediated reperfusion injury. What we found, however, is that up to 1 hour of normothermic ischemia, subsequent blood washout is still beneficial in decreasing perfusion impairment and ice formation. At 1 hour of normothermic ischemia we no longer observed any benefits from conducting blood substitution (aside from accelerating cooling).

In collaboration with Dr. R. Michael Perry at Alcor we investigated how ischemia affects the ability to chemically fix the brain. As in our cryopreservation experiments, we observed that perfusion impairment as a result of ischemia produced incomplete fixation of the brain, resulting in subsequent autolysis of the poorly fixed areas over time. These results provide a meaningful context to the current debate about the relative merits of cryopreservation or chemical fixation as pathways to long-term identity preservation because it favors cryopreservation over chemical fixation in circumstances where start of the procedure is delayed.

In addition, we also looked at the combination of chemical fixation PLUS cryoprotective perfusion and found that it is possible to completely inhibit ice formation in a chemically fixed brain, provided there are no ischemic delays between cardiac arrest and the start of chemical fixation. Interestingly, prompt chemical fixation of the rat prevents vascular leakage secondary to ischemia, which permits cryoprotective perfusion and ice free cryopreservation without edema (!) after up to 2 weeks of storage at refrigerator temperatures (~ 4° Celsius).

*"After a thorough examination of the literature and designing a lot of experiments we discovered a protocol that allowed us to successfully recover the rat from no warmer than 0° Celsius (without blood washout) by providing adequate metabolic support during cooldown and rewarming."*

Advanced Neural Biosciences further collaborated with Dr. Perry at Alcor to produce a series of electron micrographs of cortical samples of the rat brain after various periods of *normothermic* ischemia to complement the warm ischemia (room temperature) research that Alcor published in 2008. (http://www.ijcep.com/707005A.html) In 2013 and 2014 we added a series of *cold* ischemia images which includes cortical images from rats stored at water ice temperature for up to 6 months. In addition to the conventional electron micrographs we also sent a number of cortical brain samples that were subjected to a variety of interventions (e.g., straight freezing, ischemia) to 3Scan to create 3-dimensional reconstructions. To our knowledge, this is the first time that these technologies have been used for the characterization of permanent global cerebral ischemia and freezing of the brain.

This comprehensive series of images will be analyzed by experts in the field of electron microscopy and presented together in a formal paper. Dr. Perry also hopes to use these images to further develop his algorithm to characterize, infer, or predict the ultrastructural signatures of various periods of normothermic or cold ischemia. The images will also be of practical use for cryonics organizations to employ more empirical evidence to decide the maximum delays beyond which it would no longer be sensible to accept new (third party) patients after clinical death. Finally, the images can also be used as a starting point to conduct "reconstructive connectomics," i.e. the inferring of the original state of the brain from the damaged state and do "in silico" repair, which can serve as a template for biological repair.

We have also produced electron micrographs for the Cryonics Institute to characterize the ultrastructural effects of VM-1 and alternative modifications of this agent on the brain. In 2014 we produced electron micrographs for Oregon Cryonics, which uses a gentler loading protocol for VM-1 that includes more low concentration ethylene glycol steps. These images were produced using a protocol with or without an agent in the carrier solution to modify the blood brain barrier to improve penetration of the vitrification solution and reduce dehydration.

Concerns about the effects of cold ischemia sustained during transport to the facility prior to cryopreservation has renewed interest in two alternative approaches: (1) continuous perfusion of oxygenated organ preservation solutions during transport and (2) "field cryoprotection." Alcor has authorized field cryoprotection for overseas cases on the reasonable assumption that such a procedure is superior to a straight freeze. In Alcor's field cryoprotection protocol the vitrification agent is introduced in a series of discrete steps instead of a ramp, after which the patient is shipped on dry ice to the Alcor facility for further cooldown to cryogenic temperatures. In our lab we simulated this protocol and did not observe any ice formation after 48 hours storage of the brain on dry ice after cryoprotective perfusion. These findings are consistent with similar studies at 21st Century Medicine and the Cryonics Institute, including investigations of ice formation tendencies in large solutions of VM-1 and M22 at dry ice temperature.

## Current Research

Our relocation to Portland, Oregon, and our full-time employment, has allowed us to pursue a number of new, ambitious research directions.

One of our main interests in the last few years, and a prerequisite for other research projects, is whole body resuscitation from ultra-profound hypothermic circulatory arrest temperatures. We decided to pursue this model for two reasons. First, because we believe it is possible to resuscitate rats from temperatures lower than the lowest temperatures reached by Andjus, Smith, et al. While the rat and hamster resuscitation experiments from the 1950s and 1960s established the recovery of mammals from circulatory arrest at low temperatures, Smith recognized that efforts to move beyond the high subzero temperatures that were used in these experiments (down to -5° Celsius) would require extracorporeal perfusion and cryoprotectants. The increasing interest in small animal extracorporeal models, and the development of low-toxicity vitrification agents that eliminate freezing altogether, now make it possible, in principle, to go below the temperatures of those researchers and pursue real suspended animation research.

One crucial difference between hibernating and non-hibernating animals is that in the former the temperature can be lowered and heart rate and respiration will gradually decrease and eventually arrest without affecting viability of the animal. Upon rewarming cardio-respiratory activity will resume again and the animal will recover. In non-hibernating animals (such as the rat), however, lowering the temperature until circulatory arrest occurs does not produce such a synchronized condition and simply rewarming the animal is not sufficient for successful recovery from circulatory arrest. After a thorough examination of the literature and designing a lot of experiments we discovered a protocol that allowed us to successfully recover the rat from down to 0° Celsius (without blood washout) by providing adequate metabolic support during cooldown and rewarming. Our next step will be to add extracorporeal perfusion to this model, introduce a low concentration cryoprotectant, and recover the rat from high subzero temperatures.

Another important reason to pursue this model is the ability to recover organized electrical activity after cooling to ultra-profound hypothermic temperatures—the temperatures at which cryoprotective perfusion is initiated. A functional (isolated) whole brain model should be successful in lowering the temperature to 0°C and permit successful recovery of the EEG after rewarming before it can be used to study the effects of cryoprotective perfusion and cryopreservation. In the model we use in our lab this requires the re-introduction of blood or a blood substitute after the blood in the brain has been replaced with an organ preservation solution or cryoprotectant. We have screened a large number of isolated head perfusion solutions and protocols to identify a suitable protocol. At atmospheric pressures purely aqueous solutions lack the oxygen-carrying capacity for this job but we have been successful in maintaining organized electrical activity in the brain for up to 1 hour with washed red blood cells in a physiological carrier solution. Our preference, however, is to use perfluorocarbon-based emulsions which have a similar oxygen carrying capacity but lower viscosity, are easier to modify, and more practical. We currently have acquired the equipment and supplies to prepare and validate such blood substitutes. If we can successfully use these perfluorocarbon-based blood substitutes to recover electrical activity in the brain after cooling, we can combine this model with our cryobiological perfusion model and investigate the effects of cryoprotectants and cryopreservation on the whole brain with *viability* as an endpoint.

In addition to this methodologically advanced whole brain model we also use a (hippocampal) brain slice model to measure viability after cryopreservation. This model can be used to do a preliminary screen of

suitable, low toxicity cryoprotectants to be tested in the whole brain model but is also valuable to understand the effects of cryoprotectants on neural tissue without the presence of a circulatory system or blood-brain barrier. The model has been used successfully in conjunction with the potassium/sodium ratio viability assay to identify low-toxicity vitrification agents (such as M22 and VM-1) in the brain. In addition to this assay, we have identified a number of other slice viability assays that can be correlated with each other to create a compound measure of viability. Our brain slice set-up is also able to directly measure spontaneous and evoked electrical activity, which is the "gold standard" for brain slice viability.

*"We also are collaborating with Alcor to screen its comprehensive multi-modal stabilization medications protocol."*

One of our projects in 2015 is a collaboration with the Immortalist Society to bring together these viability assays and electron microscopy to validate and publish a paper about the low-cost vitrification agent VM-1 (or a lower toxicity variant).

We also are collaborating with Alcor to screen its comprehensive multi-modal stabilization medications protocol. Instead of using viability as an endpoint we look at the efficacy of these medications and solutions in improving perfusion and inhibition of ice formation after various durations of cold and normothermic ischemia. At this point we may have identified a novel approach to substantially reduce perfusion impairment and ice formation that could be a powerful tool to increase the number of cryonics cases amenable to ice-free preservation. A secondary objective of this research is to have a better understanding of which medications are most important and to establish a more compact and practical administration protocol.

## Future Research

Our whole brain cryobiology and whole body recovery models are still in early stages of development and we expect to remain quite occupied with this for years to come. We are, however, interested in a number of other topics:

1. **Cryoprotectant toxicity.** At this point there is only one lab in the world (21st Century Medicine) that can devote considerable efforts and resources toward understanding the nature and biochemistry of cryoprotectant toxicity. Since eliminating cryoprotectant toxicity is the most fundamental obstacle to reversible cryopreservation of complex organs and true human suspended animation, a greater understanding of this phenomenon is highly desirable, in particular as it pertains to the brain.
2. **Depressed metabolism.** The pharmaceutical agent Propofol is currently the only drug in Alcor's medications protocol aimed at reducing cerebral metabolism after cardiac arrest. We have no real understanding of how effective this approach is, how it compares to other anesthetics, and whether novel approaches could improve upon the (potential) benefits of Propofol.
3. **"Universal ice-free cryo-preservation."** The current situation in cryonics is that only patients with little ischemic injury can take full benefit of vitrification technologies. Our electron micrographs of cold ischemic brains have prompted some observers to speculate whether patients in which cryoprotective perfusion is no longer feasible (or is suboptimal) could benefit from immersion of the naked brain in a vitrification agent to ensure complete distribution of the agent. This possibility raises a number of practical and research questions. What are the most suitable cryoprotectants for such a protocol? How does the absence of ice formation compare to the additional damage from cold ischemia associated with such an immersion protocol? How is the brain prepared and loaded with the cryoprotectant? How will the brain be isolated and stored? etc.
4. **"Reconstructive connect-omics."** Our lab created the first 3-dimensional ultrastructural reconstructions of the damage associated with ischemia and ice formation. These images can give us some idea of the nature of the damage associated with older or suboptimal cryonics protocols. But to answer the question whether the original state can be inferred from the damaged state, more advanced methods are required, including, but not limited to, mathematical modeling of the natural and damaged state.

Our general aim is to engage in what we would call "integrated cryonics." Instead of chopping cryonics up into distinct challenges such as "ischemia," "cryopreservation" and "repair" it is often valuable to look at these issues in a "holistic" fashion. ■

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# Critical Care Research . . . . . . . . . . . . . . . . . . . . .

By Steven B. Harris, M.D

Critical Care Research (CCR) conducts a number of projects, mostly using the dog model and mostly involving cooling, which have many potential medical applications. Among these are treatment of cardiac arrest brain damage, treatment of shock-trauma, and treatment of several types of lung injury. Since cryonics involves cooling from normal body temperature down to the deep cryogenic temperature of storage (-140°C to -196°C), both CCR and its partner, 21CM have input into the design of the modern cryonics procedure, although the procedure itself is handled by other institutions.

Critical Care Research receives almost all of its support from The Life Extension Foundation.

## SUDDEN (UNEXPECTED) CARDIAC ARREST

Sudden cardiac arrest (SCA) is often defined as having the heart stop beating within an hour of any warning signs (such as chest pain) in a person who is not dying of another disease (such as cancer) and who would want to be subjected to resuscitation, if possible. SCA is quite common, killing at least 200,000 Americans a year, or at least 8% of the total of people who die each year in the US. Most cardiac arrest is due to a "heart attack" (M.I.) where a clot blocks a major heart artery and the damage induces a heart dysrhythmia.

Sudden arrest is surprisingly resistant to public health approaches. Almost half of arrests are unwitnessed, as they happen in people who are alone or asleep. In others, there is no time to get to the hospital and it requires time for the paramedics to get to the victim—time which may not be available. A person suffering cardiac arrest in the Emergency Department (ED) still only has a 66% chance of being resuscitated to normal circulation and finally discharge to home. Outside the ED this chance drops by 6% for every minute that resuscitation (return of heartbeat and blood pressure) is delayed. By 10 minutes without resuscitation outside the hospital, the chances of an adult being resuscitated and having so little brain damage as to be able to (eventually) return home, are close to zero.

Thus, while it is ***not*** true that after four to six minutes without blood flow the brain "dies" (a popular belief), it ***is*** true that after 10 minutes without blood flow, the brain of an adult has been damaged to the point that the brain will almost certainly die in a few days. It often happens, then, that heartbeat and blood pressure are restored after a cardiac arrest, yet the brain slowly deteriorates from the damage it suffered from no oxygen, until brain capillary breakdown completely stops blood from flowing to the brain, even while the heart continues to beat and the rest of the body recovers. This is the "brain death" diagnosis at which it is pointless to continue, and when the patient is pronounced legally dead, and removed from the ventilator ("life support"). At this point, cryonics perfusion of the brain does not work, either, so cryonics rescue of the brain is badly compromised. In such cases, the only solution is to quickly freeze the patient's brain, and hope that repair techniques in the future are far, far better than are needed for the "average" cryonics patient who can have cryoprotection of the brain. Perhaps strangely, there are times that initially "successful" resuscitation is worse for cryonics than no resuscitation at all.

## TREATMENT OF POST RESUSCITATION BRAIN DAMAGE

Cardiopulmonary support (CPS or CPR) such as chest compressions while waiting for paramedics to deliver an electrical defibrillation shock that restarts the heart, can increase the brain-damage-free cardiac arrest time. But CPS delivers only half of the blood the brain is used to getting, so it cannot extend the time before heart restart and brain recovery, for more than a few tens of minutes.

There are still no drug treatments for the brain damage that happens in cardiac arrest during the time there is no, or greatly reduced, blood pressure during CPR. There are many drugs that extend the time of damage-free arrest in animals, but all have failed in human trials, probably because their effect is too small to show up through the large "noise" of a trial. (There are too many variables in a trial from one person to another, including great difficulty in deciding exactly how long they have been in arrest.)

The only successful treatment for this damage discovered to date, is to cool the

brain after the heart is restarted. This effect was discovered by accident in dogs in the early 1990s. Analysis showed that dogs that happened to cool a bit (by chance) after experimental resuscitation did better. By 2000, experiments had shown the effect to be certain, and large. Just cooling the brain by 4ºC (7ºF) for 24 hours was enough to increase the damage-free arrest time by at least 50%, and sometimes 100%. The effect was something like putting a cold pack on a twisted ankle, after an injury. During the arrest these dogs did not need to be hypothermic. (Hypothermia can be applied before arrest, and works even better, but for obvious reasons, medical application is extremely limited.)

Human studies soon followed. In 2002, two prospective randomized trials in comatose resuscitated survivors of out-of-hospital cardiac arrest victims were reported. These trials used patients with return of spontaneous circulation (ROSC) but persistent coma after a witnessed episode of documented ventricular fibrillation (thus insuring that all patients had truly experienced no significant brain blood flow). Groups of untreated patients were compared with patients deliberately treated with post-arrest cooling by cold packs, or air cooling. The target was about 4ºC of cooling (7ºF) within 4 hours of ROSC, maintained for 24 hours. The outcomes of these trials were significant, with 55% vs. 39% of patients achieving an independent life in one study, and 49% vs. 26% of patients reaching this goal in the other. [1] [2]

Since these initial studies, research has focused on new and more rapid mechanisms of causing and maintaining hypothermia. Animal studies have suggested that the more rapidly the hypothermia is induced, the better its effect, but the exact parameters remain unknown. Extra-corporeal methods are very effective, but are thought to remain too invasive for resuscitation. Peritoneal and pleural lavage with ice-water have been considered. Administration of 30 ml/kg ice-cold saline solution intravenously brings about half of the desired temperature decrease, without causing pulmonary edema. A device which employs intravenous heat exchange has also become clinically available, which is able to induce the needed 7ºC temperature drop in less than two hours. It was suggested that in-hospital cooling might be useful for other rhythms and types of in-hospital cardiac arrest. [3]

*"In 2001, Critical Care Research, Inc. (CCR) published a paper [6] showing that FLL lavage was able to reduce brain temperature in dogs by a rate as fast as 0.5ºC per minute."*

## ANIMAL MODELS OF POST RESUSCITATION HYPOTHERMIA

Animal models of cardiac arrest, such as rodents and canines, have made it possible to investigate the mechanisms of modulation of post ischemic events in the cerebrum by use of direct brain biopsies and chemical analyses during the post resuscitation period. Such studies have shown that the mechanism of mild hypothermia protection of the brain in the post-ischemia period is nearly as complex as the mechanisms of damage of ischemia itself. A notable feature which stands out from these studies is that the mild degree of cooling in this type of hypothermia does *not* produce a degree of decrease in brain metabolism sufficient to explain the results, so this is not the mechanism by which the intervention proceeds. However, the mild degree of cooling does seem to substantially decrease all of the known post-resuscitation damage-cascades, including free radical release, calcium leakage, toxic neurotransmitter release, other types of inflammation reactions, and programmed cell death (apoptosis). Which of these are more important is unknown, just as the order of their importance remains unknown in normothermic ischemic damage to the brain.

Animal models have also allowed investigation of various methods of experimental cooling which would not presently be usable in clinical situations. For example, a study in 1993 by Kuboyama et al. [4] reported that a delay of only 15 minutes from resuscitation to beginning of application of hypothermia, negated the beneficial effects of cooling. Later studies extended this time, but generally found that the sooner cooling was applied, the better for the brain.

**Animal models of cooling (some of the most advanced done at CCR using lung lavage with ice cold perfluorocarbon liquids) have explored methods of cooling which cool in 1/25th of the 2 hour time needed to induce mild hypothermia (7°C below body temperature, or down to 30°C) in humans. In animals, this amount of cooling of the brain may be induced in as little as 5 minutes after insertion of an endotracheal tube.**

## LIQUID BREATHING AS EXAMPLE OF AN EXPERIMENTAL COOLING METHOD

Inhalation of water damages the lungs osmotically when the fluid is either hypo-osmotic (fresh water drowning) or hyper-osmotic (salt water drowning). However, even iso-osmotic saline causes temporary hypoxia in the lungs after the fluid is removed, via the mechanism of removal of the water soluble surfactant which allows the alveoli to remain open in the presence of great surface-tension forces. Also, saline does not carry enough dissolved oxygen to allow the medical definition of "respiration" (i.e., movement of enough oxygen and carbon dioxide exchange to support metabolism).

Perfluorocarbons (PFCs) are molecules in which all of the hydrogen atoms which occupy the non-linking surface positions of a "hydrocarbon" molecule (such as the octane molecule used in gasoline, and many others) are replaced by fluorine atoms. After this modification the PFC molecules become chemically inert. Such molecules are liquids at body temperatures, if they are heavy and complex enough. These PFC liquids do not dissolve in either water or oils. They form a new layer under them, when combined, like the drain cleaning product **"Liquid Plumr,"** and poured through standing water. But PFCs are capable of

carrying oxygen and carbon dioxide ($CO_2$), which **do** dissolve in them.

Liquid breathing with perfluorocarbon (PFC) liquids has been investigated since 1965, as a means of allowing gas exchange within the lung by means of a liquid, without removal of the critical surfactant. In the case of fluorocarbon, surfactant is not removed because it is not soluble in the PFC. The lungs can be completely filled with PFC, if it is oxygenated, in a technique called "Total Liquid Ventilation" (TLV). If a liquid ventilator machine adds and removes the PFC from the lungs, and (while outside the lungs) removes the $CO_2$ from it and adds oxygen to it, animals can be ventilated with liquid alone, without bubbles of gas in their lungs. This is the technique seen in the film ***The Abyss.*** Despite that film (where the liquid breathing scenes in humans were simulated), it has only been tried on only one human, and then only temporarily. The technique is difficult, the pressures needed close to those which cause lung damage, and the ability to dissolve $CO_2$ and thus remove it from the body is just at the edge of what is needed for dogs and humans.

In 1984 T.H. Shaffer and colleagues investigated PFCs introduced into the lungs as a method of cooling animals. The goal was not mild hypothermia, for this was not then known as a technique. However, there were other reasons to cool and warm animals, and Shaffer was able to show that the TLV cooling technique worked on anesthetized cats. The animals were not allowed to survive the experiment, however, so the long term effects of having the lungs totally full of cooled PFC liquid, were not investigated.

Another related use of PFCs developed later was a technique called Partial Liquid Ventilation (PLV) in which the lungs were filled to 1/3rd of the volume of capacity (about the amount of a normal tidal breath) with PFC, and this was allowed to remain in place while gas ventilation was then carried out "on top" of it. Ventilation was accomplished in the rest of the lung by normal gas ventilation methods. This technique could not be used for heating and cooling (since the amount of PFC was too small to affect body temperature), but it was used to open the dependent parts of the lung and assist with compromised ventilation. It underwent clinical trials in premature infants with a particular PFC called perfluorobromooctane (Perflubron™ developed by the Alliance Pharmaceutical Corporation). However, this technique was evaluated but not approved by the FDA. In these trials it did not harm the infants but didn't assist them enough to be approved.

Critical Care Research, Inc., beginning in 1997 and first patented [5] and published [6] in 2001, developed a cooling technique which was functionally a hybrid between TLV and PLV. In this technique, which we now simply call fluorocarbon lung lavage (FLL), the lungs are not completely filled with PFC, but instead filled with a volume of PFC liquid similar to only a fraction of the lung volume. This liquid is then periodically infused and removed, passing through a heat exchanger to cool it between cycles.

Initially, the PFC was also passed through a gas-exchanger in this technique, to remove $CO_2$ and add oxygen to it. However, as the volume of PFC infused was reduced in experimentation, it was found that this gas replacement was no longer necessary, and that the remainder of the lung was capable of removing $CO_2$ from the animal (and the liquid) and supplying oxygen also, if ventilated with pure oxygen gas. Thus, the gas exchanger for the PFC was not needed, and was eventually discarded.

In 2001, Critical Care Research, Inc. (CCR) published a paper [6] showing that FLL lavage was able to reduce brain temperature in dogs by a rate as fast as 0.5ºC per minute. Thus, the needed state of mild hypothermia (4ºC cooling) could be induced in less than 10 minutes (allowing for heat transfer delays). This was a factor of 12 faster than the 2 hours required by the intravenous techniques, the fastest cooling technique which had been reported by any method.

Cardiac bypass techniques were known to be able to cool in excess of 1°C per minute, but circulatory bypass involves gaining access to major blood vessels (usually the femoral arteries and veins in the groin) and there is a necessary delay time to initiate bypass after an emergency. It also requires a skilled vascular surgeon. PFC lung lavage, by contrast, could be initiated as soon as an endotracheal tube was in place in the patient's "windpipe", and in theory could be done in the field, by paramedics.

## DEVELOPMENT OF LUNG LAVAGE

Unlike other experimental labs, CCR normally allowed many of its experimental animals (canines) to survive long term, after the procedure. It was found by this protocol that an asthma-like syndrome was produced by high-rate lavage with many PFCs, but that this did not become appreciable until 24 hours after lavage-cooling. It was never fatal if lavage was done carefully enough under low pressure, but did cause the animals difficulty breathing for as long as week after the procedure. Eventually, as with all PFC procedures, the PFC evaporates and disappears from the lungs, and the animals recover completely.

Over the course of the last 14 years, CCR made an exhaustive study of the necessary parameters of lung lavage to minimize the post-lavage syndrome. These include investigation of 10 different candidate PFC fluids (in some cases this was forced by the manufacturers discontinuing them, as none of them are made for medical use). CCR research has also explored the optimal lavage volume, timing, temperature, and method of delivery and mode of removal. Because PFC is heavy and resists suctioning, the optimal methods to introduce and remove it turned out to require a dedicated apparatus and a very finely tuned method of use. This proved quite formidable, and the CCR machine required complete re-design four times.

At the same time this development was taking place, Lance Becker at Argonne National Laboratories had been working with tiny ice spheres which could be suspended in PFC as a slurry, and delivered to the lungs. The phase change as the ice melts provides an extra degree of cooling power per volume of lavage which PFC alone does not, but on the other hand, the saline generated in this way also presumably dissolves some lung surfactant. Becker reported initial tests of this system in euthanized animals, but did not end up

developing it further. As in many of these systems, since the animal was not allowed to survive, we do not know the long term effects of the procedure.

## CHALLENGE OF BECKER, ET AL TO PRODUCE ULTRA-RAPID BRAIN COOLING IN A LARGE ANIMAL

In 2007, Dr. Becker and Joshua W. Lampe published a paper [7] in the journal ***Expert Review of Medical Devices***, in which they challenged experimenters to design a cooling system which would lower brain temperature by 4°C in the first 5 minutes of application. They wrote:

> *"The induction of mild hypothermia, lowering body temperature by 4°C, is gaining acceptance as an acute therapy for the treatment of hypoxia and ischemia following cardiac arrest and many life-threatening injuries. When hypothermia is used following ischemia (as opposed to before ischemia), it needs to be performed rapidly for the greatest benefit, preferably within* **5 min.** *A simple model reveals that this poses a significant bioengineering challenge as the rate of heat transfer is severely limited, owing to a relatively confined fundamental parameter space. Current methods of cooling include external cooling devices, such as cooling blankets or ice bags, which are simple to use, and relatively inexpensive but slow. Internal cooling has the best ability to cool more rapidly but current devices are more invasive, costly and most are still not able to provide cooling within the rapid 5-min interval."*

Lampe and Becker go on to a discussion of theoretical amounts of heat which can be removed by a number of methods, including cardiac bypass, but note that the surgery for this takes at least 1 hour to implement. They conclude:

> *"Hypothermia of 3-4°C must be achieved within 5 min for maximum protection when required after a period of ischemia. The human body is well designed to prevent heat loss, creating a significant bioengineering challenge in a relatively confined fundamental parameter space. The minimum temperature and the maximum volume of coolant that can be introduced into the body are significant engineering obstacles."*

In reading Lampe and Becker's article, CCR realized that they had seriously miscalculated the amount of heat which would need to be removed during rapid cooling, due to their inexperience with the decrease in effective heat capacity of the body, which occurs during very rapid cooling of the blood. CCR's previous experiments had found that only a 60% to 70% "core" mass (which includes the brain, fortunately) is cooled in the first few minutes of cooling, and that if cooling continues past 5 minutes, the extra heat to be extracted in the 6th and 7th minute will make up for the later "re-equilibration" which takes place within the animal or human as the cold core and brain are re-warmed by the periphery (which does not initially cool as fast as they do). The differential cooling of the thermal core during very fast cooling allows a temporary window in which only part of the body (including the brain) needs to be cooled, with the catch-up period of re-equilibration later, used for compensatory cooling.

Since Lampe and Becker had also assumed that the heat capacity of the body is that of an equivalent amount of water (it is actually only 70% as large) their estimates of needed heat extraction were off by a factor of 70% times 70% (a reciprocal factor of 2). Also, their estimations of the amount of cold fluorocarbon which could be delivered to the lungs, was incorrect. At CCR it was realized that the challenge set by Lampe and Becker had already nearly been met.

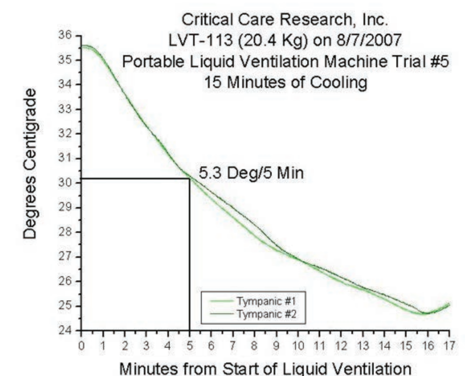
## WORK ON PORTABLE HIGH CAPACITY LUNG LAVAGE DEVICE

Beginning in 2007 CCR contracted with outside engineering consultants to provide a suitable high capacity (high suction) machine, which would be completely portable (able to run from batteries) and also able to cool the body more than 4°C, should this be required. In designing this machine, CCR made use of the high-capacity commercial "plate-type" heat exchangers already available on the commercial market for other uses (these are capable of nearly 600 watts/degree gradient of heat transfer between ice-water and PFC). CCR then discarded the bulky peristaltic pumps and replaced them with inexpensive commercial fluid diaphragm pumps, which proved far superior, and able to run on low voltage D.C.

With the superior suction capabilities of this device, combined with its ability to provide a constant supply of perfluorocarbon cooled to 2ºC (from a reservoir containing only 7 liters of fluid) CCR performed a series of 30 lung-lavage dog experiments in 2007 and mid 2008. The first results were encouraging enough to apply for a preliminary patent on the device in September 2007, and a full patent application was filed (Platt, Battiano, Harris) for the device in September 2008 [8].

A graph from one of the early experiments with this device, after design parameters had been partly adjusted, illustrates performance:

This graph shows that Lampe and Becker's stated goal (which they thought impossible without a direct application of a phase-change substance such as ice to the body) of 4ºC drop in 5 minutes has been exceeded with the device, which provided 5.3ºC drop in the first 5 minutes of application . In addition, in this experiment, CCR was able to cool the animal's brain and heart to 25ºC (77ºF), in just 16 minutes.




Later experiments with the device show that 6-7 minutes of application of 60 ml/kg/min lung-lavage with ice-cold perfluorcarbon (2ºC) results in at least 4ºC

drop in brain temperature in the first 5 minutes, and that application of lavage for more than 6 minutes results in a permanent body core temperature drop of 4ºC. All of these results are novel, and should be of great importance to the resuscitation community.

Note that all these problems (and benefits!) of having the core of the animal substantially cooler than the periphery, only happen in extreme rates of cooling, such as are not seen in any other cooling techniques applicable to the field in emergencies. However, they now serve as part of the basic technique at CCR.

The post-lavage asthma syndrome which is exacerbated by the wrong choice of PFC, and by use of the wrong techniques during lavage (which result in needless overpressures), has also been overcome by CCR. This syndrome is not known by other groups, because CCR presently has the only large-animal model of lung lavage, where the animals are allowed to survive long enough to develop later lung reactions. (CCR maintains its own dog colony, with all animals bred "in house" since 2003).

The present extreme cooling technique causes little or no asthmatic reaction in dogs, and they are clinically comfortable, and survive.

The following graph shows the performance of this machine in detail, when used for 5.3 minutes to cool a dog's brain by 4.8ºC in 6.75 minutes, slightly less than the best performance seen in the graphs above. This dog has been fully instrumented, so that the instantaneous change in central venous and arterial blood temperatures (in the aorta and inferior vena cava) can also be seen. Note the excursions in both venous and arterial blood with each of the 40 lavages (one lavage every 8 seconds) in this experiment. Blood temperatures fall as low as 29.2ºC (84.6ºF) in this animal which begins at a temperature of 37ºC (98.6ºF). At the end of the experiment, equilibration of venous and arterial blood can be seen, followed by equilibration of the entire blood pool as a whole within the core of the animal, as represented by the brain temperature (this happens after about 700 seconds or a bit less than 12 minutes, when blood and brain temperatures begin to track in parallel). Note that blood temperature still exceeds brain/core temperature by about 0.4ºC at 900 seconds (15 minutes) because at this time, blood is still carrying heat from the warm periphery of the animal to the cooled core tissues, which include the brain (that is, full body tissue and compartment heat equilibration is not complete).

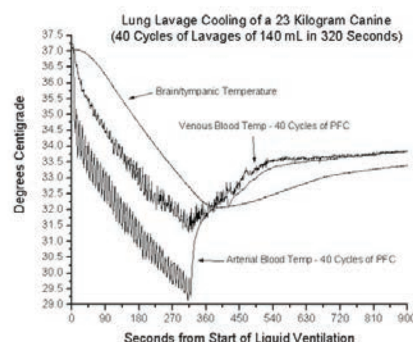



**Figure: Animal Lavaged with Device in Patent, 2008. In the Figure above temperatures in the venous system, arterial system, and brain are recorded. Lung lavage is given for 5.3 minutes. Brain temperature does not begin to drop rapidly until 45 seconds, which reflects a circulation delay, as the cooling of the arterial blood is seen to be immediate. However, the overshoot effect occurs after the procedure is stopped, and the minimal brain temperature does not occur until almost 6.75 minutes (a minute and a half after the procedure has stopped), and it is 32.2ºC, which is a 4.8º drop from a beginning temperature of 37ºC (98.6ºF). Over the next hour the brain will warm to 33.7 ºC (3.3ºC drop), as the cold core of the body equilibrates with the warmer exterior (about 20% of the total thermal "mass"). In general, continuous lavage of about 6 minutes is required to guarantee a 4ºC drop at 5 minutes, as well as a permanent brain temperature drop of 4ºC even after body and brain equilibration, one hour after liquid ventilation cooling has been stopped. To cool the body and brain permanently by 4ºC requires an early and instantaneous brain cooling of about 5.5ºC, which later develops into a permanent "whole body" drop of 4ºC after equilibration of the cold thermal core with the warmer (but less well perfused) periphery. Thus, the brain must be rapidly cooled by 5.5ºC to retain a 4ºC drop.**

## THE FUTURE OF POST RESUSCITATION HYPOTHERMIA

The technique of post-resuscitation cooling, even with simple ice-bags in the Emergency Department, has been slow to catch on. Problems include logistical difficulties and even lack of advertising to move information (there are no large pharmaceutical companies involved). A 2005 survey of 256 emergency room physicians found that despite official ILCOR endorsement of this technique for Advanced Life Support (ALS) in 2003, only a quarter of these physicians were using the technique on their resuscitated patients.[9] The reasons given were that the technique was difficult or slow, as well as a technically correct but misguided assertion that the ALS guidelines did not incorporate it (these guidelines had not been updated since 2000, at that time, so they had not been able to incorporate cooling recommendations).

*"The post-lavage asthma syndrome which is exacerbated by the wrong choice of PFC, and by use of the wrong techniques during lavage...has also been overcome by CCR."*

Moreover, simple ice-bag techniques as used in emergency departments are often ineffective at cooling. A recent (2009) study reported in *Journal Watch Emergency Medicine* reported that post-resuscitative cooling of 287 patients with ventricular fibrillation arrest, using ice packs, cooling blankets and cooling pads, reached the target temperature of 32ºC-34ºC in only 65% of patients. [10] Despite this, the group found that treatment increased survival to hospital discharge from 39% to 54% and

favorable neurological outcome rate from 15% to 35%. They wrote: "…it is time for [Emergency Departments] to implement hypothermia protocols for comatose survivors of cardiac arrest."

These and other results have continued to amaze physicians who had believed that the traditional 5 to 10 minutes of warm circulatory arrest time (without chest compression) was the limit, after which brain death was certain. A recent *Wall Street Journal* article quoted one "early-adopter" of the post-resuscitation cooling protocol [11]:

> *"We've had patients who have been stone-cold out for least 20 minutes—we know that for sure—and they've come back normal or nearly normal," says Michael Mooney, a cardiologist who heads the therapeutic hypothermia program at Minneapolis Heart Institute. An early adopter of the cooling technique, the cardiology practice has treated more than 140 patients since 2006 and says 52% have survived, compared with single digits historically; of those, about 75% have had a "favorable neurologic recovery," including many who report a full return to normal.*

The difference between 10 minutes and 20 minutes is particularly important, for this is the average paramedic response time for a large fraction of urban areas in the country. Nor are physicians sure that 20 minutes is the limit for resuscitation, as these studies are still being done with mostly conventional methods of cooling with ice packs and esophageal ice water lavage, which takes at least several hours.

### THE FUTURE OF LUNG LAVAGE

Critical Care Research, Inc. has already solved many of the key problems in inducing very rapid brain mild hypothermia (4ºC drop by 5 minutes of lavage, sufficient heat removal for a permanent 4ºC drop after equilibration, after 6 minutes of lavage), with minimal post procedure lung reaction in the following days. Remaining, however, are the difficult and expensive clinical trials which must occur in humans.

Dogs, with more delicate and slightly larger lungs (for their body size) than humans, are a good model for lung lavage. Although it may be possible to cool a dog slightly more quickly than a human by this method, at the same time, if the technique does not damage dog lungs at a given pressure, it probably will not damage the tougher human lungs. Even so, for safety's sake, initial clinical trials in humans will need to be carried out at a fraction of the maximal rates and pressures which have been achieved in canines.

Lung lavage remains to be tested after resuscitation, in an actual large-animal model of brain ischemia and circulatory arrest. ■

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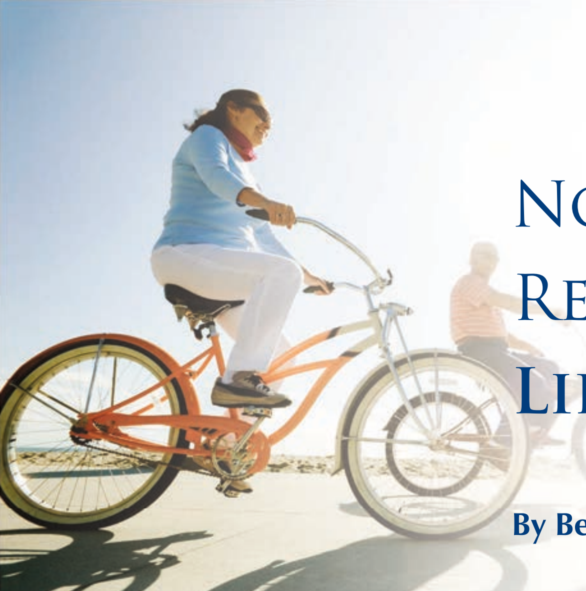

# Non-Cryonics Research Recently Funded by the Life Extension Foundation

**By Ben Best, Director of Research Oversight, Life Extension Foundation**

Although the Life Extension Foundation (LEF) contributes substantially to cryonics and cryobiology-related research, much LEF funding is devoted to anti-aging research (including anti-cancer research). LEF supports dozens of scientists seeking to meaningfully extend the healthy human lifespan through their unique research initiatives.

**Drs. Gorbunova and Seluanov** are a husband-and-wife team at the University of Rochester in Rochester, New York. Both scientists are devoted to finding means to reduce aging and cancer so as to extend human lifespan.[1] Dr. Gorbunova discovered that the sirtuin SIRT6 could more than triple the repair of DNA damage.[2] Increased levels of SIRT6 has been shown to extend the lifespan of male mice 10-15%.[3] Because DNA damage can lead to both aging and cancer, Dr. Gorbunova has been looking for molecules which will stimulate SIRT6 activity. Dr. Gorbunova was denied government funding on the grounds that there are other mechanisms of DNA repair besides what SIRT6 stimulates. Grants from the LEF have enabled Dr. Gorbunova to continue her work on SIRT6 activation of DNA repair.

Dr. Seluanov has the second-largest naked mole rat colony in the world. Although mice usually die of cancer, cancer has never been reported in a naked mole rat.[4,5] Naked mole rats live about ten times longer than mice without evidence of aging or age-related diseases.[6] Dr. Seluanov was denied funding from the government on the grounds that the genome of the naked mole rat had already been sequenced to discover the basis of naked mole rat cancer-resistance and longevity. Without funding from LEF, Dr. Seluanov would have been in danger of losing his naked mole rat colony. Although Gorbunova and Seluanov contributed to analysis of the naked mole rat genome,[7] this information was insufficient to explain the cancer-resistance and longevity of the naked mole rat.

On July 18, 2013 Seluanov and Gorbunova made the cover of the prestigious journal NATURE with their discovery that high molecular weight hyaluronan (hyaluronic acid) in naked mole rats protects them from cancer.[8] Naked mole rat hyaluronan has five times the molecular weight of the hyaluronan in humans or mice. Hyaluronan is found in skin products. In other mammals, hyaluronan contributes to wound healing.[9] But in the naked mole rat, the high molecular weight hyaluronan causes cancer cells to stop growing.[8] A few years earlier, Dr. Seluanov had discovered that naked mole rat tissue causes cancer to stop growing,[10] but he had not understood the reason.

With LEF funding, the couple subsequently discovered that protein synthesis is four times more accurate in naked mole rats than in mice.[11] Many neurodegenerative diseases such as Alzheimer's Disease and Parkinson's Disease are associated with protein mis-folding, which is likely in part due to errors in protein synthesis.[12] Precision synthesis of proteins by naked mole rats contributes to their cancer-resistance and longevity.

Both the hyaluronan discovery and the protein synthesis fidelity discovery caused the prestigious journal SCIENCE to name the naked mole rat "Vertebrate of the Year" for 2013.[13] The August 2014 issue of NATURE REVIEWS: GENETICS featured a review as a cover story on that subject that was primarily authored by the two scientists.[14] NATURE REVIEWS: GENETICS is the foremost scientific journal (highest impact factor) on the subject of heredity and genetics. LEF was acknowledged as a funding source for the review.

> *"Joao Pedro de Magalhaes, PhD...used funds from LEF to sequence the genome of the bowhead whale, the longest-living mammal, which lives more than 200 years."*

**Joao Pedro de Magalhaes, PhD**, (Senior Lecturer/Associate Professor, Institute of Integrative Biology, Liverpool University, Liverpool, United Kingdom) used funds from LEF to sequence the genome of the bowhead whale, the longest-living mammal, which lives more than 200 years.[15] With more cells in their bodies, larger animals would be expected to have higher rates of cancer.[16] Even tall humans have a higher cancer risk than short humans, independent of all other risk factors.[17] But a variety of anti-cancer mechanisms allows larger animals to

avoid the greater cancer risk that would otherwise accompany their greater size.[18] Dr. Magalhaes's laboratory is uniquely equipped to analyze the genome of the bowhead whale to determine the means by which it achieves such longevity while avoiding cancer.[19] With funding from LEF, Dr. de Magalhaes was able to complete genome sequencing of the bowhead whale, and publish an analysis of the genome.[20]

**John Furber, MSc**, (CEO, Legendary Pharmaceuticals, Gainesville, Florida) attends many conferences dealing with the biology of aging every year, and he is therefore very well-known among biomedical gerontologists. He has designed a rejuvenation experiment which LEF is funding. Lysosomes are the garbage disposal/recycling centers of biological cells. Lysosomes contain enzymes that digest cellular waste products into reusable components.[21] But with time, lysosomes accumulate enzyme-resistant age-pigment molecules known as lipofuscin.[22] Some neurons in the brain can contain up to 75% lipofuscin.[23] Lipofuscin emits toxic free radicals. Cells loaded with lipofuscin cannot be expected to function very well, so lipofuscin may contribute to the maladies of old age. Furber would like to rejuvenate cells by removing the lipofuscin. By doing an extensive search of scientific literature, he identified some chemicals which induce cells to export lysosomal lipofuscin out of the cell.[24] With LEF funding he is experimenting with these chemicals in the hope removing lipofuscin from cells without causing harmful effects when the lipofuscin enters the bloodstream.

**Justin Rebo, MD**, (Research Scientist, SENS Foundation, Mountain View, California) is using Life Extension Foundation funding to investigate in detail why shared blood circulation between genetically identical mice of different ages rejuvenates the old mouse, and makes the young mouse biologically older.[25,26] Blood from a young mouse contains more functional white blood cells and stem cells[27] as well as fewer inflammatory proteins than blood from an old mouse.[28] Dr. Rebo's objective is to determine with precision all of the positive components of young blood and all of the negative components of old blood. With this information, he will develop strategies to enhance the positive components as well as to block or remove the negative components. He is hopeful that his results will be ready for clinical application in only a few years.

*"Justin Rebo, MD... is using Life Extension Foundation funding to investigate in detail why shared blood circulation between genetically identical mice of different ages rejuvenates the old mouse, and makes the young mouse biologically older."*

LEF has been funding the South Florida Bone Marrow/Stem Cell Institute (Principal Investigator **Dipnarine Maharaj, MD, FACP**) for clinical trials treating cancer victims with white blood cells from young donors. A future grant is planned for an experiment to use blood components from young donors to relieve an elderly person from generalized symptoms of age-related frailty and ill health.

University of California at San Francisco (Principal Investigator, **Cynthia Kenyon, PhD**) has received funding from LEF to screen for drugs that increase stress resistance in nematode worms. There is good evidence that greater stress resistance will increase lifespan, healthspan, and resistance to aging-related diseases (with application to humans).[29] Dr. Kenyon gained fame for her discovery that the lifespan of nematode worms could be doubled by genetic manipulation. This led to subsequent discovery that genetic manipulation could also extend the life and health of rodents.

**Robert Shmookler Reis, PhD**, Professor at the University of Arkansas for Medical Sciences, Little Rock, Arkansas. The research of Dr. Reis has been focused on the influence of genetics on longevity and the diseases of aging. Although we have known for the better part of a century that calorie restriction slows aging in rodents[30] and that lifespan is largely under genetic control in many or all species,[31,32] it is only in the last two decades that the genes and pathways regulating lifespan have been discovered. In 1988 mutation in the age-1 gene was shown to increase the average lifespan of nematode worms by 40–65%[33] In 1993 Cynthia Kenyon showed that *daf-2* mutations could double the lifespan of nematode worms.[34] These genes were later found to lie in the same genetic pathway, which when manipulated in mice can stretch their lifespan by half.[35] Two decades after the first long-lived mutant in age-1 was characterized,[33] Dr. Reis found that more thorough elimination of this gene's PI3K gene product can actually extend nematode life span tenfold.[36] Dr. Reis believes that this benefit can extend far beyond worms. Suppression of PI3K in mouse heart muscle slows many measures of heart aging and improves their overall survival.[37] Crippling just one of the normal two copies of PI3K in all tissues of the mouse is bad for juvenile mice but improves fitness, metabolism, and survival after maturity.[38] Humans who live past age 100 show an inherent genetic bias that produces the same effects.[39] Dr. Reis seeks to identify the molecules that are directly affected by the most beneficial genetic modification, and to find drugs that can knock out PI3K and mimic the life-extending benefits observed in previous studies. Nematode worms are an ideal biochemical laboratory for life span studies of this nature, but Dr. Reis also experiments with human cells and mice.

In the Fall of 2014 the LEF increased its funding for Androcyte, Inc. for analysis of the genomes of centenarians and supercentenarians (persons over age 110). Such long-lived persons possess rare protective genes that could benefit everyone from the debilities of aging and aging-associated diseases. The chief scientific officer of Androcyte is **Dr. George Church**, the world-famous Harvard geneticist who is at the forefront of the new CRISPR gene-editing technology. To date, only one human (Timothy Ray Brown) has been documented to be cured of the HIV virus. Brown was suffering from

both leukemia and AIDS. Brown received a hematopoietic stem cell transplantation that fortunately gave him two copies of the CCR5 gene that blocks HIV.[40] If CRISPR technology can be perfected and made safe, not only could AIDS be cured, but genes from supercentenarians could protect everyone from aging-associated diseases, like Alzheimer's Disease.

LEF is funding **Howard Chang, MD, PhD**, at the Stanford School of Medicine. Dr. Chang has been studying gene expression (epigenetic) changes in stem cells that occur with aging. He has identified a number of pathways that affect stem cell epigenetics, and is seeking drugs that will alter those pathways. Rejuvenation of stem cells will improve wound healing as well as recovery from a variety of disease conditions. The elderly often die of immune system failure,[41] which could be prevented by stem cell rejuvenation.[42] Dr. Chang was an Ellison Foundation Scholar until his grant expired. Without LEF funding he could not have continued his research aimed at intervening in the aging process. Aside from the Life Extension Foundation, it is difficult to find sources of funding for aging research. The National Institutes of Health only funds research on specific diseases and the Ellison Foundation has ceased funding anti-aging research.

Another former Ellison Foundation Scholar being funded by LEF is **James Shorter, PhD**, Associate Professor of Biochemistry and Biophysics at the University of Pennsylvania. Dr. Shorter has discovered that heat shock proteins can reverse the aggregation of toxic proteins, including those causing Alzheimer's Disease, Huntington's Disease, Parkinson's Disease, ALS ("Lou Gehrig's disease"), among others. With funding from the Life Extension Foundation he has made progress in developing heat shock proteins that reverse the amyloid-beta protein aggregation that is believed to initiate Alzheimer's Disease. He is now focusing his attention on the aggregation of the tau protein that kills neurons in the later stages of Alzheimer's Disease.[43,44,45,46]

Cancer cells have the ability to multiply without restraint, unlike other cells which are limited in their ability to multiply because of their shortening telomeres. Cancer cells usually use telomerase enzyme to multiply, which is why so many researchers have been attempting to stop cancer by telomerase interference. But cancer cells have another trick to multiply, known as ALT (Alternate Lengthening of Telomeres).[47] Very few researchers have been working on ALT, but if researchers find a way of blocking telomerase in cancer cells, cancer cells will increasingly use ALT to multiply. Cancer cells utilizing ALT to lengthen telomeres can be identified by ALT-associated bodies in the cell nucleus,[48] but ALT researchers have been hampered by the time-consuming and expensive methods required to study ALT. LEF funded **Dr. Haroldo Silva** of the SENS Foundation to develop an improved method of screening for ALT, which should boost the entire field of ALT research.

**Dr. Victoria Belancio** of the Tulane School of Medicine has been researching retrotransposons ("jumping genes") that cause genome instability. Retrotransposon activity increases with age, resulting in increased cancer and other aging-associated diseases.[49] With funding from LEF, Dr. Belancio has established that shift workers are at greater risk for retrotransposon-induced cancer because of light at night reducing melatonin secretion.[50]

**Orn Adalsteinsson, PhD**, who is chairman of the Life Extension Foundation Scientific Advisory Board, supervises a number of anti-cancer projects and clinical trials funded by LEF, many of which are conducted in the Bahamas. Only a couple of these will be described.

The rapid expansion in the use of Positron Emission Tomography, or PET scans, to obtain metabolic information about cancer lesions can provide oncologists and their patients with extremely valuable diagnostic and treatment management information. PET scans use an injected radioactive tracer material like fluorodeoxyglucose (FDG) to produce functional imaging that can help differentiate benign from malignant masses, evaluate tumor stage, monitor response to therapy and detect tumor recurrence in a variety of malignancies.[51] Coupled with the precise anatomical imagery produced by computerized tomography, FDG PET/CT can give rapid and accurate information about tumor size, location and rate of growth.

As useful as PET imaging can be, statistical errors can at times result in "false negative" and "false positive" reporting. Other issues that may trigger errors include improper PET scanner calibration with errors which can at times result in false patient body weight, and the variability in FDG uptake depending on the elapsed time from when the radiotracer was injected into the patient. But the most egregious errors are perhaps due to incomplete or inconsistent scan interpretations caused by inadequate training and a lack of overall standards for the quantified reporting of results. Incorrect PET scans are common today and can result in improper treatments for cancer patients.

*"In the Fall of 2014 the LEF increased its funding for Androcyte, Inc. for analysis of the genomes of centenarians and supercentenarians (persons over age 110)."*

Working with radiologist Richard Black, MD, the International Strategic Cancer Alliance adopted invaluable PET reporting practices in its LEF-supported laser-assisted immunotherapy breast cancer trial. Dr. Black has interpreted more than 80,000 PET/CT studies, and his methodology for an across-the-board upgrade in PET scan reporting should be incorporated at the national level to provide oncologists and their patients with the full potential PET technology has to offer. The five key features of Dr. Black's approach will assure that oncologists receive the same kind and quality of information on each and every scan, regardless of who interpreted the scan, or where it was taken.

1. **Quantitative Reporting:** Standardized uptake values, or SUV readings are collected for every

object of concern in the scan, not just narrative descriptions.

2. **Reproducible Reporting:** SUV readings are standardized to an area of normal homogenous tissue in the liver to generate a corrected SUV for every area of concern. The correction factor allows different experts using different equipment to obtain similar results.

3. **Index Lesion Focus:** "Hotspots" indicating tumor activity must be monitored from one study to the next to enable rapid and accurate measurements of changes over time or in response to therapy.

4. **Comparative Readings Mandate:** PET scan reporting must make reference to the size, SUV, and other features of an index lesion(s) from previous scans, obligating the current radiologist to request those studies for a side-by-side comparison.

5. **Image Snapshots of Index Lesions:** Allows the ordering physician to visualize the areas of abnormality, rather than relying solely on a written report.

Dr. Black presented his initial findings at one of LEF's Scientific Advisory Board Meetings in 2012; his presentation can be viewed on the Life Extension website at the following URL: www.LEF.org/PET-CT

The critical need to develop superior cancer imaging tools cleared a major hurdle in December 2012, when a U.S. pharmaceutical giant agreed to sell the shelved research and development rights to Combidex, a revolutionary magnetic resonance imaging (MRI) contrast agent. Combidex-enhanced scans can detect metastatic cancer lesions too small to be seen by traditional PET/CT imaging.[52]

Life Extension Foundation continues to be a strong advocate of Combidex since helping with the negotiation of the sale of the Combidex technology package to Radboud University Medical Center in the Netherlands in 2012. In 2013, world-renowned radiologist Jelle Barentsz, MD, with the assistance of LEF through Orn Adalsteinsson, has begun the process of preparing the launching of multi-country research trials, which will ultimately lead to new license applications, a commercialized product and widespread patient access.

Combidex (ferumoxtran-10) is composed of a simple sugar compound, dextran, and superparamagnetic iron oxide, or USPIO.53 These extremely small iron crystals (25-50 nanometers in diameter), become powerfully magnetized when exposed to the magnetic field of an MRI scanner. The injected Combidex contrast fluid is taken up selectively by the macrophages (scavenger cells) that are primarily found in lymph nodes and other inflammatory tissue.[53,54]

Dr. Barentsz is one of the few physicians in the world to have worked extensively with Combidex technology, predominantly in prostate cancer cases. In one study, Dr. Barentsz and his team compared traditional CT scans and Combidex-enhanced MRI lymphangiography (MRL) for 375 prostate cancer patients, 16% of whom had lymph node metastases. CT imaging detected only 34% of the positive nodes, while Combidex MRL identified a remarkable 82%. The diagnoses were microscopically confirmed by either a lymph-node dissection or a needle biopsy. The study group concluded that Combidex-enhanced MRL is 96% accurate, and can eliminate the need for highly invasive surgical lymph node dissections.[55]

Combidex scans have also been used to successfully evaluate patients with cancers of the uterus,[56] head and neck,[57] kidney,[58] breast,[59] and liver.[60] ■


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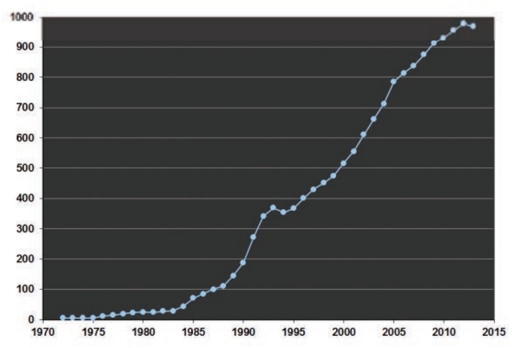
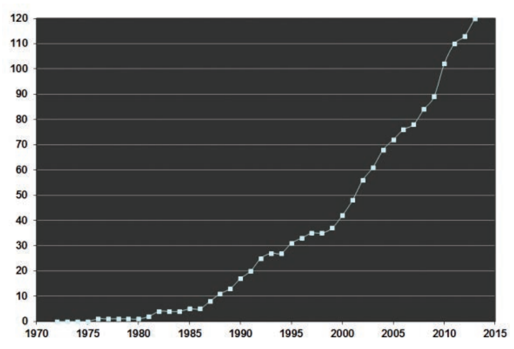
# Membership Statistics

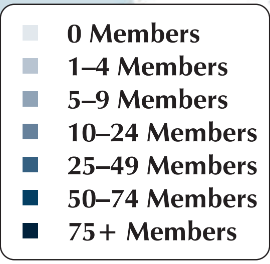
| 2015 | JAN | FEB | MAR | APR | MAY | JUN | JUL | AUG | SEP | OCT | NOV | DEC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Members | 1016 | | | | | | | | | | | |
| Patients | 134 | | | | | | | | | | | |
| Associate | 151 | | | | | | | | | | | |
| Total | 1301 | | | | | | | | | | | |



*Number of Alcor members*



*Number of Alcor patients*




## International

| Country | Members | Patients |
|---|---|---|
| Australia | 10 | 3 |
| Canada | 43 | 2 |
| Germany | 5 | 0 |
| Hong Kong | 1 | 0 |
| Israel | 1 | 1 |
| Italy | 3 | 0 |
| Japan | 3 | 0 |
| Mexico | 4 | 0 |
| Monaco | 1 | 0 |
| Netherlands | 2 | 0 |
| New Zealand | 2 | 0 |
| Norway | 1 | 0 |
| Portugal | 4 | 0 |
| Singapore | 1 | 0 |
| Spain | 3 | 1 |
| Thailand | 3 | 1 |
| United Arab Emirates | 1 | 0 |
| United Kingdom | 23 | 2 |
| TOTAL | 111 | 10 |

# ARE YOU GETTING Curcumin's BENEFITS?

***Curcumin*** is the health-promoting trace compound derived from the Indian spice **turmeric**. But not all turmeric is alike.

The curcumin found in the vast majority of dietary supplements is derived from turmeric that is **nutritionally inferior**.

Why? Almost all growers harvest turmeric at the point when the turmeric root turns its signature yellow color, but *before* it has fully matured.

The turmeric root requires more time in the ground for highly beneficial phytonutrients called ***curcuminoids*** and ***sesquiterpenoids*** to attain peak concentrations.

**Life Extension**®'s **Super Bio-Curcumin**® derives from turmeric that is grown with organic practices, cultivated to maturity, then specially transported and processed to preserve and deliver the root's most complete nutritional profile.

In recent studies comparing the effects of standard curcumin against turmeric extracts comparable to **Super Bio-Curcumin**®, researchers observed:[1,2]

- Nearly twice the support for **immune** health.
- Approximately twice the support for **inflammatory** issues.
- Almost double the **antioxidant** support.

A separate study indicated that an antioxidant-rich curcumin extract[3] provided powerful support for heart health.

## Unrivaled Potency and Absorbability with BCM-95®

Curcumin is neither absorbed nor *retained* well in the blood, which is another challenge facing those who wish to maximize its benefits.

The highly popular **Super Bio-Curcumin**® uses **BCM-95**®, a patented, *bioenhanced* preparation of curcumin. It has been shown to reach up to **7 times higher concentration** in the blood than standard curcumin.[4]

The graphs on this page illustrate that one **400 mg** vegetarian capsule per day of **Super Bio-Curcumin**® supplies the equivalent of **2,500 mg** of commercial curcumin supplements.

A bottle containing 60 vegetarian capsules of **Super Bio-Curcumin**® retails for $38. If a member buys four bottles, the price is reduced to only **$26.25** per bottle.

**Item # 00407**


**References**

1. *Int J Pharmacol*. 2009;5(6):333-45.
2. *J Food Nutr Res*. 2009;48(3):148-52.
3. *Arch Gerontol Geriatr*. 2002;34:37-46.
4. *Indian J Pharm Sci*. 2008 Jul-Aug;70(4):445-9.
5. Bioavailability study of BCM-95® in rats. Orcas International Inc. 2006.


**CAUTION:** Do not take if you have gallbladder problems or gallstones. If you are taking anti-coagulant or anti-platelet medications, or have a bleeding disorder, consult your healthcare provider before taking this product.


**Bio-Curcumin**® and **BCM-95**® are registered trademarks of Dolcas-Biotech, LLC.
U.S. Patent Nos. 7,883,728, 7,736,679 and 7,879,373.


**To order Super Bio-Curcumin® call 1-800-544-4440 or visit www.LifeExtension.com**

## How Much Curcumin Are You Absorbing?

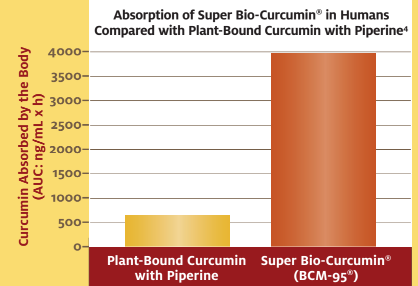



**Chart 1.** Super Bio-Curcumin® (BCM-95®) showed 6.3 times greater bioavailability (absorption and sustainability over 8 hours) in humans compared with plant-bound curcumin with piperine (as measured by the area under the curve [AUC] in a plot of blood levels against time, that is, the total amount of curcumin absorbed by the body over 8 hours).

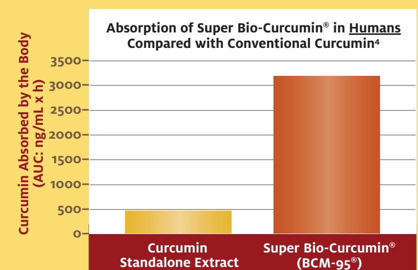



**Chart 2.** Super Bio-Curcumin® (BCM-95®) showed 6.9 times greater bioavailability (absorption and sustainability over 8 hours) in humans compared with conventional curcumin (as measured by the area under the curve [AUC] in a plot of blood levels against time, that is, the total amount of curcumin absorbed by the body over 8 hours).

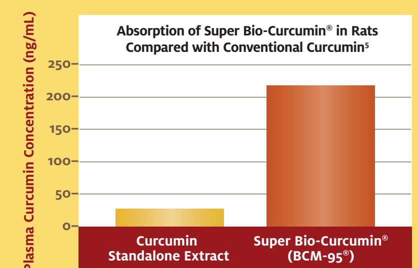



**Chart 3.** Bioavailability in rats fed with BCM-95® is 7.8 times higher than conventional curcumin.


These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease.

## CRISPR Gene Edits Human Stem Cells Precisely and Efficiently

A powerful "genome editing" technology known as CRISPR has been used by researchers since 2012 to trim, disrupt, replace or add to sequences of an organism's DNA. Now, scientists at Johns Hopkins Medicine have shown that the system also precisely and efficiently alters human stem cells. Previous research has shown that CRISPR (third generation gene editing) can generate genomic changes or mutations through these interventions far more efficiently than other gene editing techniques, such as TALEN (2nd generation gene editing). The researchers compared the ability of both genome editing systems to either cut out pieces of known genes in iPSCs or cut out a piece of these genes and replace it with another. As model genes, the researchers used JAK2, a gene that when mutated causes a bone marrow disorder; SERPINA1, a gene that when mutated causes an inherited disorder that may cause lung and liver disease; and AAVS1, a gene that's been recently discovered to be a "safe harbor" in the human genome for inserting foreign genes.

NextBigFuture
12 Jan. 2015
http://nextbigfuture.com/2015/01/crispr-gene-edits-human-stem-cells.html

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## Providing a Clearer Picture of Nanotechnology's Full Potential

A new tool capable of carrying out simultaneous nano-sized measurements could soon lead to more innovative nanotech-based products and help boost the EU economy. Indeed the tool, developed by scientists cooperating through the EU-funded UNIVSEM project, has the potential to revolutionize research and development in a number of sectors, ranging from electronics and energy to biomedicine and consumer products. Nanotechnology has led to new materials—such as graphene—and microscopic devices that include new surgical tools and medicines. Up until now however, nanotech R&D has been hampered by the fact that it has not been possible to achieve simultaneous information on 3D structure, chemical composition and surface properties. This is what makes the UNIVSEM project, due for completion in March 2015, so innovative. By integrating different sensors capable of measuring these different aspects of nano-sized materials, EU scientists have created a single instrument that enables researchers to work much more efficiently.

Community Research and Development Information Service (CORDIS)
12 Jan. 2015
http://cordis.europa.eu/news/rcn/122251_en.html

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## Cooling Brain Protein Could Aid Search for Alzheimer's Treatment

Scientists have found a mechanism that kicks in when the body is cooled and prevents the loss of brain cells, and say their find could one day lead to treatments for brain-wasting diseases such as Alzheimer's. Studying mice, the researchers were able to simulate the effects of body cooling and pick apart the workings of a so-called "cold-shock" protein in the brain, RBM3, which has previously been linked with preventing brain cell death. "We've known for some time that cooling can slow down or even prevent damage to brain cells, but reducing body temperature is rarely feasible in practice (because) it's unpleasant and involves risks such as pneumonia and blood clots," said Giovanna Mallucci who led the research. "By identifying how cooling activates a process that prevents the loss of brain cells, we can now work toward finding a means to develop drugs that might mimic the protective effects of cold on the brain." Scientists already know that lowering body temperature can protect the brain.

Kate Kelland, Alison Williams / Reuters
14 Jan. 2015
http://www.reuters.com/article/2015/01/14/us-health-brain-cooling-idUSKBN0KN25J20150114

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## $4 Million in Funding to develop Artificial General Intelligence

AGI Innovations Inc, (www.AGi-3.com) an R and D company focused on advancing Artificial General Intelligence (AGI) has secured $4 million in funding to support research on long-term AGI development. AGI is the AI discipline concerned with developing systems with human-like cognitive abilities such as general learning, reasoning, and problem solving. AGI Innovations Inc, also known as AGi3, was formed early in 2014 to continue research originally spearheaded by Adaptive A.I. Inc (a2i2), an AGI R and D company formed in 2001. On completing its first generation AGI engine in 2008, a2i2 launched. Peter Voss, founder of these companies, explains that while commercialization helped to validate the AGI approach taken by the company, it also totally shifted focus away from general AI to providing specific solutions to its customers. "Our AGI research was essentially halted. Fortunately, we now have a new dedicated and funded company focused entirely on long-term AGI development" Voss said.

NextBigFuture
21 Jan. 2015
http://nextbigfuture.com/2015/01/agi-innovations-secures-4-million-in.html

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## Scientists Upload a Worm's Mind into a Lego Robot

A humble roundworm is leading the race in artificial intelligence, showing that it may be possible one day to upload our brains to a computer. Called the Open Worm Project, the research brings together scientists and programmers from around the world with the aim of recreating the behavior of the common roundworm (*Caenorhabditis elegans*) in a machine. The open source project recently had its first major breakthrough when its software—modeled on the neurons of the worm's nervous system—independently controlled a Lego robot. The machine's sensors, without any prior programming, made the robot behave in a similar fashion to C. elegans, approaching and backing away from obstacles or stimulated by food. "We've been working on it for four years and while we have a lot more to achieve it's been the most surprising project I've been involved in," project coordinator Stephen Larson told CNN. "It's certainly exceeded my expectations." The project aims to digitally model the worm entirely in a virtual environment; creating a robot with an elastic body complete with stretchy muscles.

Gant Daily
21 Jan. 2015
http://gantdaily.com/2015/01/21/scientists-upload-a-worms-mind-into-a-lego-robot/

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## Researchers Unboil Egg Whites

Researchers at the University of California Irvine (UCI) along with colleagues in Australia have successfully unboiled egg whites, meaning that they have managed to quickly and inexpensively restore the proteins in the egg to their original state. When an egg is boiled, the heat causes the proteins in the egg to tangle and clump together. This is the process that turns the gelatinous liquid inside the egg into a solid. Unboiling is probably not something that most people will ever need to do in the kitchen. However, in science the same basic process has the potential to reduce the cost of cancer treatment, food production and other important areas of the biotechnology industry, which is worth $160 billion globally. "Yes, we have invented a way to unboil a hen egg. In our paper, we describe a device for pulling apart tangled proteins and allowing them to refold. We start with egg whites boiled for 20 minutes at 90 degrees Celsius and return a key protein in the egg to working order," said Gregory Weiss, UCI professor of chemistry and molecular biology & biochemistry.

Justin Beach, *National Monitor*
25 Jan. 2015
http://natmonitor.com/2015/01/25/researchers-uncoil-an-egg-with-profound-implications-for-food-medicine-and-biotech/

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# A Roadmap to Resuscitation

Successful rejuvenation of cryonics patients will require three distinct technologies: (1) A cure for the disease that put the patient in a critical condition prior to cryopreservation; (2) biological or mechanical cell repair technologies that can reverse any injury associated with the cryopreservation process and long-term care at low temperatures; (3) rejuvenation biotechnologies that restore the patient to good health prior to resuscitation. OR it will require some entirely new approach such as (1) mapping the ultrastructure of cryopreserved brain tissue using nanotechnology, and (2) using this information to deduce the original structure and repairing, replicating or simulating tissue or structure in some viable form so the person "comes back."

The following list is a list of landmark papers and books that reflect ongoing progress towards the resuscitation of cryonics patients:

Jerome B. White, "**Viral-Induced Repair of Damaged Neurons with Preservation of Long-Term Information Content,"** Second Annual Conference of the Cryonics Societies of America, University of Michigan at Ann Arbor, April 11-12, 1969, by J. B. White reprinted in *Cryonics* 35:10 (October 2014), 8-17.

Michael G. Darwin, **"The Anabolocyte: A Biological Approach to Repairing Cryoinjury,"** *Life Extension Magazine* (July-August 1977):80-83. Reprinted in *Cryonics* 29:4 (4th Quarter 2008),14-17.

Corey Noble, **"A 'Realistic' Scenario for Nanotechnological Repair of the Frozen Human Brain,"** in Brian Wowk, Michael Darwin, eds., *Cryonics: Reaching for Tomorrow,* Alcor Life Extension Foundation, 1991.

Ralph C. Merkle, **"The Molecular Repair of the Brain,"** *Cryonics* 15(January 1994):16-31 (Part I) & *Cryonics* 15(April 1994):20-32 (Part II).

Ralph C. Merkle, **"Cryonics, Cryptography, and Maximum Likelihood Estimation,"** First Extropy Institute Conference, Sunnyvale CA, 1994.

Aubrey de Grey & Michael Rae, **"Ending Aging: The Rejuvenation Breakthroughs That Could Reverse Human Aging in Our Lifetime."** St. Martin's Press, 2007

Robert A. Freitas Jr., **"Comprehensive Nanorobotic Control of Human Morbidity and Aging,"** in Gregory M. Fahy, Michael D. West, L. Stephen Coles, and Steven B. Harris, eds, *The Future of Aging: Pathways to Human Life Extension,* Springer, New York, 2010, pp. 685-805.

Chana de Wolf (now Phaedra), **"Reconstructive Connectomics,"** *Cryonics* 34:7 (July 2013), 26-28.

# MEETINGS

## ABOUT THE ALCOR FOUNDATION

The Alcor Life Extension Foundation is a nonprofit tax-exempt scientific and educational organization dedicated to advancing the science of cryopreservation and promoting cryonics as a rational option. Being an Alcor member means knowing that—should the worst happen—Alcor's Emergency Response Team is ready to respond for you, 24 hours a day, 365 days a year.

Alcor's Emergency Response capability includes specially trained technicians and customized equipment in Arizona, northern California, southern California, and south Florida, as well as many additional certified technicians on-call around the United States. Alcor's Arizona facility includes a full-time staff, and the Patient Care Bay is personally monitored 24 hours a day.

## ARIZONA

### FLAGSTAFF:

Arizona without the inferno. Cryonics group in beautiful, high-altitude Flagstaff. Two-hour drive to Alcor. Contact eric@flagstaffcryo.com for more information.

### PHOENIX VALLEY OF THE SUN:

This group meets monthly, usually in the third week of the month. Dates are determined by the activity or event planned. For more information or to RSVP, visit http://cryonics.meetup.com/45/ or email Lisa Shock at lisa@alcor.org.

### AT ALCOR:

Alcor Board of Directors Meetings and Facility Tours—Alcor business meetings are generally held on the first Saturday of every month starting at 11:00 AM MST. Guests are welcome to attend the fully-public board meetings on odd-numbered months. Facility tours are held every Tuesday and Friday at 2:00 PM. For more information or to schedule a tour, call Marji Klima at (877) 462-5267 x101 or email marji@alcor.org.

## CALIFORNIA

### LOS ANGELES:

Alcor Southern California Meetings—For information, call Peter Voss at (310) 822-4533 or e-mail him at peter@optimal.org. Although monthly meetings are not held regularly, you can meet Los Angeles Alcor members by contacting Peter.

### SAN FRANCISCO BAY:

Alcor Northern California Meetings are held quarterly in January, April, July, and October. A CryoFeast is held once a year. For information on Northern California meetings, call Mark Galeck at (650) 969-1671, (650) 534-6409 or email Mark_galeck@pacbell.net.

## FLORIDA

Central Florida Life Extension group meets once a month in the Tampa Bay area (Tampa and St. Petersburg) for discussion and socializing. The group has been active since 2007. Email arcturus12453@yahoo.com for more information.

## NEW ENGLAND

### CAMBRIDGE:

The New England regional group strives to meet monthly in Cambridge, MA—for information or to be added to the Alcor NE mailing list, please contact Bret Kulakovich at 617-824-8982, alcor@bonfireproductions.com, or on FACEBOOK via the Cryonics Special Interest Group.

## PACIFIC NORTHWEST

A Yahoo mailing list is also maintained for cryonicists in the Pacific Northwest at http://tech.groups.yahoo.com/group/CryonicsNW/.

### BRITISH COLUMBIA (CANADA):

The contact person for meetings in the Vancouver area is Keegan Macintosh: keegan.macintosh@me.com.

### OREGON:

The contact person for meetings in the Portland area is Aschwin de Wolf: aschwin@alcor.org

See also: https://www.facebook.com/portland.life.extension

## ALCOR PORTUGAL

Alcor Portugal is working to have good stabilization and transport capabilities. The group meets every Saturday for two hours. For information about meetings, contact Nuno Martins at n-martins@n-martins.com. The Alcor Portugal website is: www.alcorportugal.com.

## TEXAS

### DALLAS:

North Texas Cryonauts, please sign up for our announcements list for meetings (http://groups.yahoo.com/group/cryonauts-announce) or contact David Wallace Croft at (214) 636-3790 for details of upcoming meetings.

### AUSTIN/CENTRAL TEXAS:

We meet at least quarterly for training, transport kit updates, and discussion. For information: Steve Jackson, 512-447-7866, sj@sjgames.com.

## UNITED KINGDOM

There is an Alcor chapter in England. For information about meetings, contact Alan Sinclair at cryoservices@yahoo.co.uk. See the web site at www.alcor-uk.org.

**If you are interested in hosting regular meetings in your area, contact Alcor at 877-462-5267, ext. 113. Meetings are a great way to learn about cryonics, meet others with similar interests, and introduce your friends and family to Alcor members!**

# What is Cryonics?

Cryonics is an attempt to preserve and protect human life, not reverse death. It is the practice of using extreme cold to attempt to preserve the life of a person who can no longer be supported by today's medicine. Will future medicine, including mature nanotechnology, have the ability to heal at the cellular and molecular levels? Can cryonics successfully carry the cryopreserved person forward through time, for however many decades or centuries might be necessary, until the cryopreservation process can be reversed and the person restored to full health? While cryonics may sound like science fiction, there is a basis for it in real science. The complete scientific story of cryonics is seldom told in media reports, leaving cryonics widely misunderstood. We invite you to reach your own conclusions.

# How do I find out more?

The Alcor Life Extension Foundation is the world leader in cryonics research and technology. Alcor is a non-profit organization located in Scottsdale, Arizona, founded in 1972. Our website is one of the best sources of detailed introductory information about Alcor and cryopreservation (www.alcor.org). We also invite you to request our FREE information package on the "Free Information" section of our website. It includes:

- A fully illustrated color brochure
- A sample of our magazine
- An application for membership and brochure explaining how to join
- And more!

**Your free package should arrive in 1-2 weeks.** (The complete package will be sent free in the U.S., Canada, and the United Kingdom.)

# How do I enroll?

Signing up for a cryopreservation is easy!

**Step 1:** Fill out an application and submit it with your $90 application fee.
**Step 2:** You will then be sent a set of contracts to review and sign.
**Step 3:** Fund your cryopreservation. While most people use life insurance to fund their cryopreservation, other forms of prepayment are also accepted. Alcor's Membership Coordinator can provide you with a list of insurance agents familiar with satisfying Alcor's current funding requirements.
**Finally:** After enrolling, you will wear emergency alert tags or carry a special card in your wallet. This is your confirmation that Alcor will respond immediately to an emergency call on your behalf.

Not ready to make full arrangements for cryopreservation? Then **become an Associate Member** for $10/month (or $30/quarter or $120 annually). Associate Members will receive:

- *Cryonics* magazine by mail
- Discounts on Alcor conferences
- Access to post in the Alcor Member Forums
- A dollar-for-dollar credit toward full membership sign-up fees for any dues paid for Associate Membership

To become an Associate Member send a check or money order ($10/month or $30/quarter or $120 annually) to Alcor Life Extension Foundation, 7895 E. Acoma Dr., Suite 110, Scottsdale, Arizona 85260, or call Marji Klima at (480) 905-1906 ext. 101 with your credit card information. You can also pay using PayPal (and get the Declaration of Intent to Be Cryopreserved) here: http://www.alcor.org/BecomeMember/associate.html

**Call toll-free TODAY to start your application:**

**877-462-5267 ext. 132 • info@alcor.org • www.alcor.org**

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