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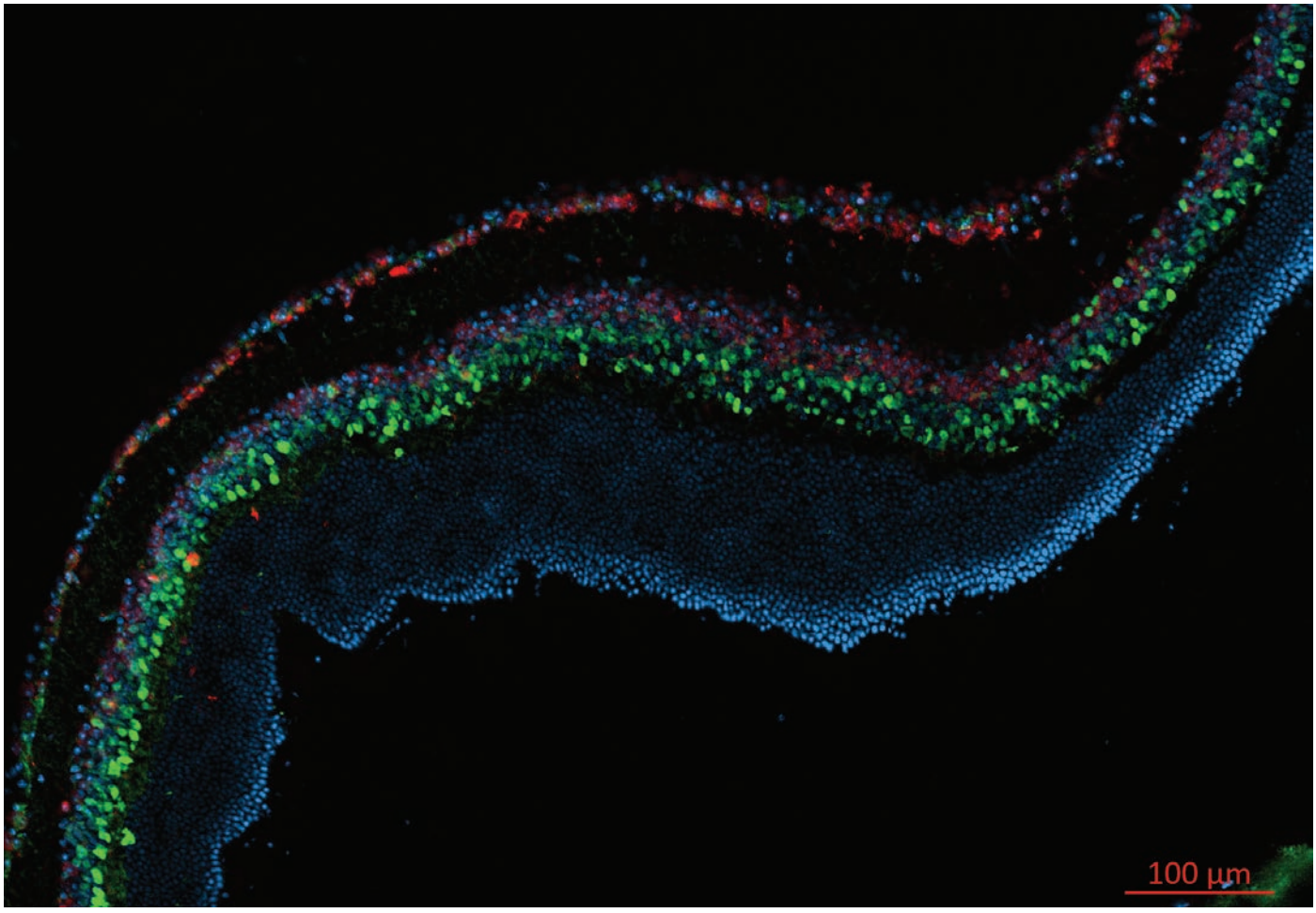

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Address correspondence to:
Cryonics Magazine
7895 East Acoma Drive, Suite 110
Scottsdale, Arizona 85260
Phone: 480.905.1906
Toll free: 877.462.5267
Fax: 480.922.9027

Letters to the Editor welcome:
aschwin@alcor.org

Advertising inquiries:
480.905.1906 x113
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Scholar Profile: Roman Bauer, Ph.D.

By Nicole Weinstock

Dr. Roman Bauer

The recent coronavirus pandemic has spurred a number of comparisons and historical coverage of the 1918 influenza pandemic that swept the globe just over a century ago. Though both events inspire(d) a sense of solidarity and collaboration across the sciences, this latest test of innovation carries the distinctive force of computational modeling. For computational biologist and cryopreservation researcher, Dr. Roman Bauer, this moment serves to underscore the power of computational models in a broad range of biological research, including cryonics:

> It can be brains, cryopreservation of biological tissues, the spreading of pandemics – if we can collect data, model based on this data, make predictions, and then test those predictions, then we can get a much better insight on what's going on.

With a robust background in computer science and neuroinformatics, Bauer has dedicated his career to the advancement of computational modeling in experimental biology. He is best known for his initiation and ongoing leadership of the BioDynaMo cohort. A free accessible software, the BioDynaMo platform allows computer science experts and non-experts alike to create, run, and visualize 3D agent-based biological simulations from a simple laptop. If Bauer's vision comes to fruition, the platform will eventually become a formidable nexus of agent-based modeling where users will be able to freely share models and test for reproducibility around the globe.

Bauer has relied on BioDynaMo for a number of past projects, in addition to his current research focus on the cryopreservation of neural tissue. Based on results thus far, he and his team are optimistic about the value of their computational tools in optimizing cryopreservation protocols, a potentially significant benefit for cryonics.

Discovering Neuroinformatics

Dr. Bauer was born in Zürich, Switzerland to a Turkish artist and art therapist, and a Swiss computer programmer. "I think my current work as a computational biologist combines the two main features of my parents' work quite well," Bauer observes, "namely, creativity and the usage of computers."

Though he grew up in a small town in northeast Switzerland, he returned to urbanity to pursue his B.Sc., followed by his M.Sc. and Ph.D., at the acclaimed public research university, ETH Zürich. Bauer arrived with a deep-seated interest in artificial intelligence (AI), but the university had no official AI course. He pursued the Computational Science and Engineering (CSE) course, focusing on robotics and theoretical physics. An important turning point emerged when he discovered a collaboration between ETH Zürich and the University of Zürich, called the Institute of Neuroinformatics. Considered a cutting-edge collaboration, it was founded in late 1995, just a couple years after the field of its naming was coined.

The union of neuroscience and information science, neuroinformatics is principally concerned with the development of modern computational tools and databases capable of managing, analyzing, modeling, and sharing vast amounts of high-dimensional neuroscience data.[1,2] It relies heavily on computational models: mathematical models generated by computers that simulate and study complex systems ranging anywhere from weather to infectious diseases.[3] In the case of neuroinformatics, these systems relate to the brain.

Given his enduring interest in AI, Bauer saw a unique opportunity at the institute, which eventually supported his CSE master's thesis. It investigated how complex networks develop in the brain, drawing from the theory of morphogenesis by British mathematician, Alan Turing. Though widely regarded as the father of theoretical computer science and artificial intelligence, Turing also made significant contributions to biology and chemistry. In his 1952 article, *The Chemical Basis of Morphogenesis*, Turing discussed the biological processes that give rise to an organism's particular shape and form, e.g., stripes, spots, tails, etc.[4] He coined the term "morphogen" to refer to the specific chemical agents that initiate or dictate morphogenesis.[5] Bauer's thesis examined these morphogens to determine whether or not they could exist in the brain – they can and there is strong evidence that

they do – using computers to simulate the development of biologically plausible networks.

"My master's thesis was really the first time that I directly looked at AI from a biological point of view," says Bauer. He was drawn to the interdisciplinary nature of the study and resolved to lean into it. In the fall of 2009, he began his Ph.D. in Neuroinformatics under the guidance of Professor Rodney Douglas, a pioneer in neuromorphic computation and co-founder of the institute.

Creating BioDynaMo

Bauer's Ph.D. stemmed from an interesting comparison between neurons and genes. He explains:

> The human brain has 86 billion neurons, and about 10^{15} synapses, so it's extremely large and very specifically connected...We have about 20,000 protein coding genes, and in total about 60,000 genes, which is much smaller than the number of neurons or synapses in the brain. Somehow this genetic code manages to specify our brain, and computers are a great way to explore how this works.

That was the objective of Bauer's Ph.D. thesis, which culminated in the first model of cortical neural network self-organization in 3D space. This "first" was significant not just for the outcome but for the means as well. Bauer used a specific class of computational modeling called agent-based modeling (ABM) or individual-based modeling (IBM) – something that has become a foundational tool in his current work.

Though quite complex, ABMs are models that simulate a certain phenomenon over time. They are programmed to show multiple parts or "agents" of a system in addition to their respective interactions. The agent interactions within the model are then governed by a series of if/then rules set by the programmer.[6] "For instance," Bauer explains, "in the brain we know that neurons communicate with one another via electrical impulses. We also know that they communicate with each other via substances that diffuse into space. Agent-based models allow you to take all of those interactions into account on all different levels."

A powerful tool, ABMs are particularly effective in modeling complex biological systems where small variations tend to cause dramatic system changes; however, they are not without their drawbacks. The principal disadvantage of ABMs is that they are very computationally demanding. That is why, despite dating back to the 1940s in concept, they did not gain traction until the 90s (when computers became more widespread and much more powerful).[7]

Bauer was frustrated by the resource limitation of such a promising avenue for neuroinformatics. He began to envision a software that could harness the power of ABM on a single computer, but still within a reasonable amount of time. He knew that kind of advancement could not be achieved alone. It would require outside expertise in not just computational excellence, but more precisely, computational *efficiency*. Who had the resources and reputation for the job?

In 2013, Bauer began a Research Associateship under Professor of Neuroinformatics, Marcus Kaiser, at Newcastle University (NU) in England. Concurrent with his research, he continued to chip away at his ABM software idea. With encouragement from his friend and co-founder of SCImPULSE Foundation, Dr. Marco Manca, Bauer pitched it to the European Organization for Nuclear Research (CERN).

The birthplace of the World Wide Web, CERN is an international collaboration of 23 member states that unites some of the most brilliant scientific minds to conduct world-class research in fundamental physics.[8] The organization uses the world's largest and most powerful particle accelerator, the Large Hadron Collider (LHC). The accelerator's 16.5-mile ring-shaped form straddles the border of its French-Swiss headquarters.[9]

CERN accepted Bauer's proposal and in 2018 the first working version of the aptly named open-source biology dynamics modeler "BioDynaMo" was launched. Thanks to their efforts, and those of a growing number of collaborators like the University of Cyprus and GSI Darmstadt, BioDynaMo can now simulate tens of millions of neurons from a simple laptop around the globe. Though that may seem tremendous – and it is, relatively speaking – Bauer reminds us that it is still short of the 86 billion neurons that exist in the brain. Nevertheless, he estimates that if the project continues at its current rate of progress, this goal will be fulfilled in approximately seven years.

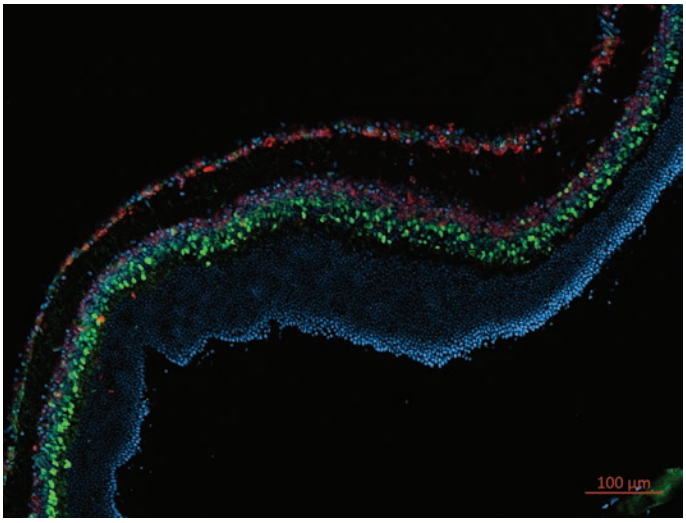
For Dr. Bauer, BioDynaMo is also a way to share the benefits of agent-based models with biologists who lack a strong computational background. "They also have great ideas and should be able to establish their ideas on a computer to see whether they agree with the experiments that they conduct." He adds, "I think it's an important task for people like me to help people that don't have much insight into computer programming to use it in such a way that it helps their own research, their own understanding."

Perhaps the most potent of possibilities for BioDynaMo is its potential to affect reproducibility: the degree of agreement between the results of the same experiment run by different people in different locations with different instruments.[10] Bauer cites a rather shocking 2016 survey by *Nature* magazine showing that 70% of surveyed researchers were unable to reproduce another scientist's experiments. Over 50% of them shared that they also failed to reproduce their own experiments.[11] If BioDynaMo can grow to be a central hub for agent-based model sharing, Bauer believes that reproducibility concerns can be heavily mitigated, perhaps even eradicated:

In computational biology, people often use different software, different data of course, and different ways to model it. But if agent-based modeling can provide a platform where people can freely upload how they created their models, and then other people can download that and run it on their computers locally, that's the ultimate goal. Then we would have reproducibility.

Modeling Retinal Cryopreservation

BioDynaMo has continued to be a vehicle for change in Dr. Bauer's career in the time since its inception. In the fall of 2016, he began a Medical Research Council (MRC) Fellowship to computationally model the development of the retina using BioDynaMo. Though laity may often refer to the eye and brain with implied separation, the retina is an extension of the brain, a thin membrane formed by layers of neural cells that line the back of the eye. Despite it forming a proportionately small amount of neural tissue, the retina still holds many mysteries, Bauer observes. "[The retinal neurons] are all electrically active and they already do computation, but we don't really understand exactly how the retina computes the inputs that it receives."



The retina is a neuronal tissue composed of multiple layers, as highlighted in this detailed figure provided by Dr. Bauer.

Bauer believes that cultivating a better understanding of the retina is an important prerequisite to mastering the brain as a whole. His is a relatively fresh perspective as compared to most cryonics research, which has traditionally focused on the latter. Retinal modeling could also provide life-changing insights into neurodevelopmental disorders like Leber congenital amaurosis and retinopathy of prematurity. Knowing what causes them, and when and how they arise could enable medical intervention to reduce or prevent their growth entirely.

During this MRC investigation, Bauer crossed paths with a number of stem cell biologists working with retinal organoids,

Dr. Bauer during a presentation on the cryopreservation of biological tissues.

such as his NU colleague Professor Majlinda Lako. The organoid, an artificially grown mass of cells or tissue derived from pluripotent stem cells to resemble a miniature human organ, allows researchers to study human development and model disease in a way that has otherwise only been possible with non-human model organisms.[12]

Like many advances in-the-making however, organoid research faces a number of obstacles. Storage, Bauer says, is a principal challenge. A retinal organoid may seem small, as compared to a kidney or lung organoid, but it still takes close to nine months to grow to completion. Any change in protocol – introducing a new drug, for example – could take weeks or even months to manifest, making it not only difficult for researchers to pinpoint cause-and-effect, but to avoid having to scrap a partially grown organoid entirely. For this reason, Bauer explains, the ability to cryopreserve a retina would be a huge asset to the field of organoid development.

With this in mind, Bauer applied for a United Kingdom Research and Innovation (UKRI) Fellowship with the Engineering and Physical Sciences Research Council (EPSRC). Through a joint affiliation with NU's Bioscience Institute (formerly the Institute of Genetic Medicine) he proposed the use of BioDynaMo once more to simulate and model the retinal cryopreservation process. He was awarded the fellowship and began research with his current NU team in 2018.

This foray into the world of cryopreservation has been eye-opening to Bauer. With so many years of computational experience at his back, he was surprised by the prevalence of

empirical research in the field. Computers play a relatively small role. "That's what my fellowship focuses on. It's about improving and bringing in this computational component to facilitate those different parameters that are currently just based on a trial and error process."

So far, Dr. Bauer and his team have taken important strides in this direction. For example, with the help of BioDynaMo, they have been able to simulate how cells change over time during freezing: how water concentration is affected, how cells shrink over time, and how cryoprotective agents influence this process. "Ultimately, we would like to have a computational tool set that cryopreservation scientists can use to render their work more efficient."

Computational Biology for Cryonics

One of the greatest scientific weak points in cryonics is the inability to show successful brain preservation post-resuscitation. Bauer shares the sentiments of many when he says, "We need to be sure that the information in our brain, which defines our personality, is not lost. The only way to show this is by demonstrating that the circuits and the electrical activity that they give rise to can still be reproduced after cryopreservation."

Through his vision of BioDynaMo and its potential to positively impact protocols and reproducibility, Dr. Bauer makes a strong argument for the promise of computational toolsets to cryonics. The ability to create a hypothesis, to test, compare and run models, and ultimately, choose the one that agrees most with an experiment – this could usher in a new age of faster and cheaper cryonics research. Proving the widespread reproducibility of cryopreservation models could also lend more credibility to the field, thereby encouraging more cryonics memberships. A stronger membership base could herald a number of favorable shifts for cryonics, from cost structures, to legal influence, to field preservation capabilities, and more.

Dr. Bauer at the 1st Alcor New York Symposium in the fall of 2019.

Dr. Bauer and his NU team are currently in the process of publishing their findings from the last two years, so he is unable to provide more specifics. Nevertheless, he is excited to hint at promising news. "All I can say is that by using these [computational] tools it seems like we can pretty dramatically decrease the number of experiments that are needed to cryopreserve the cells." Looking forward, he hopes to apply his current research to various other tissues of the mouse brain. As he points out, mice have a brain architecture similar to humans, are close in genetics to humans, and cost-effective – an ideal case scenario for testing the efficacy of neuropreservation. ■

To contact Dr. Roman Bauer about his research or professional opportunities, please contact him directly at roman.bauer111@gmail.com and/or visit his personal website, www.romanbauer.net. For more on the open-source biology dynamics modeler, BioDynaMo, go to www.biodynamo.org.

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Q&A with Roman Bauer

1. What role do you think computational genomics can play in the repair and revival of cryonics patients?

Computational methods that help gain insights into the information stored in the DNA will not only be important but also necessary for revival. This is because the cryopreservation process will surely inflict injury to at least some parts of the brain. These injuries might be difficult or impossible to repair without genetic information. Insights into how the genetic code specifies the development of neural tissue will help regenerate these; however, additional computational tools beyond those used in computational genomics are also required. This is because we are not only the result of our genes, but also our environment. The brain in particular is highly plastic and dynamically changes during development and aging in response to the plethora of stimuli and environmental factors. Hence, computational genomics is necessary though not sufficient for the repair and revival of cryonics patients.

2. It has been estimated that more than half of all genes express themselves in the brain. Does this mean that some ultrastructural details from a patient's brain can be inferred from the genome alone?

Many structural aspects of the brain are encoded in the genome. For instance, we know that there are gender-specific differences in several key brain structures (Wierenga et al., *Cerebral Cortex*, 2018). The genome alone however, cannot explain the brain's detailed organization. During brain development, complex interactions take place that are governed by the genome and many other processes. For instance, cells secrete substances that diffuse in extracellular space, and these influence the growth of neurons. Small variations in the molecular concentrations of these guidance cues (e.g. due to changes in the physical properties of extracellular space), which are not entirely determined by the DNA, affect the intricate structure and therefore also the function of neurons. Importantly, so-called "epigenetic" factors strongly influence brain development. These influences are not solely determined by the genome. Along those lines, what our mothers eat and drink during pregnancy, what music they listen to, their hormonal levels etc., all have an impact on our brain structure and function.

3. What role can computational biology play in inferring the healthy condition of the brain from the cryopreserved condition of the brain?

Given the sheer number and complexity of cells in the brain, computational tools are absolutely necessary to capturing the cryopreserved condition and reproducing its healthy state. In the future, these technologies could be optimized in such a way that they reproduce the condition of the brain as accurately as possible before cryopreservation. This task requires methods to analyze, store, and model extremely large amounts of data on multiple scales. Advanced machine learning techniques can be used along these lines, as is the case in the great study of (de Wolf et al., Rejuvenation Research, 2020) where deep learning was employed.

Looking at the big picture, computational biology can play two crucial roles in this endeavor: firstly, it can provide methods for the analysis of the cryopreserved condition in order to identify defects that occurred during cryopreservation. The other role is the storage of information from the healthy condition before cryopreservation. Such an *in-silico* replica would be of major value and current technology already allows the non-invasive measurement of brain structure at high resolution. For instance, 7-Tesla MRIs can achieve a resolution of approximately 0.5 millimetres, and recently 11.7 Tesla scanners have been built. The processing and analysis of such data is currently a core subject in computational neuroscience, a subfield of computational biology.

4. How can computational models of the brain be used to assist in in-situ molecular repair of the patient?

The capability for molecular repair is arguably the holy grail of revival. If one can re-establish the molecular composition of the brain as it was before cryopreservation, then one has the toolset necessary to reproduce consciousness. Clearly, such technology lies in the distant future, but there are research directions that are relevant now and that can aid in reaching this goal.

For instance, one particularly promising approach would be the usage of nanorobots. This is because the brain is made up of an extremely large number of molecules (a single synapse includes about 5,000 neurotransmitter molecules, and there are about 10^{15} synapses in the human brain). Hence, it would be better to repair it using a large number of distributed, small entities, rather than a large device. The scientific field that deals with the question of how to organize large numbers of interacting elements or agents (i.e. swarm intelligence) is still in its infancy. Such systems are highly nonlinear, and we currently lack a well-defined formal system to specify, model, and predict the behavior of such swarms. This is a topic that is relevant to my current research, as the agent-based simulator BioDynaMo can be used to model and study very large systems of agents as well as their interactions within biological tissues.

5. What kind of studies can be done to further develop the concept of information-theoretic death?

A lot of work can and should be done, because this is a question of major importance to human existence. It is relevant to so many fields, including medicine, technology, and philosophy; however, I think that at the core of this question lies the concept of consciousness. We need to better understand what consciousness is, because it is consciousness that matters, not necessarily the aliveness of the underlying biological tissue. Would you be willing to be revived in the future in an unconscious but metabolically active biological substrate? I don't think so. We require a better understanding of the physical basis of consciousness. This mind-body problem is of course very old, and many bright minds have thought about it.

Nevertheless, now is a very exciting time to address this problem. Recent technological advances allow us to address questions of consciousness much more objectively than ever before. We can measure brain activity dynamics at high spatial and temporal resolution. We can also stimulate the brain in a controlled and consistent manner. Such experiments have given rise to many important insights into brain function. They also allow us to study the link between neural dynamics and consciousness on a quantifiable level. By simplifying and modeling these quantitative measurements, one can get a better understanding of how neurons produce thoughts and feelings. There is still a long way to go, but the last few years have allowed researchers to reduce the (previously immense) gap between the fields of psychology and neuroscience.

Richard Feynman once said, "What I cannot create, I do not understand." There is a lot of wisdom in this sentence, especially with regard to the quest to understand consciousness. Theories, mathematical equations or even computer simulations alone are not sufficient to demonstrate consciousness. The philosopher John Searle captures it nicely when he writes, "Nobody supposes that the computational model of rainstorms in London will leave us all wet." Studies on the creation of neurally-inspired robots that express complex behavior are important lines of research. Of course, a robot that behaves in a way that appears conscious does not necessarily possess consciousness. Nevertheless, I think such research can help gain insights on certain aspects of consciousness, and also information-theoretic death.

6. Cryoprotectants have poor blood-brain-barrier penetration. What kind of technologies can study the interaction of the BBB and cryoprotectants?

This is a very interesting topic, not only for cryonics but also for many biomedical applications. Hence, it is not surprising that substantial research aims to overcome the blockage of large-molecule drugs by the BBB. Indeed, many promising methods exist, and further research is required to test these possibilities. For instance, focused ultrasound can be used to open the BBB and enhance the penetration of the central nervous system by therapeutic substances (Chen, Wei and Liu, *Front Pharmacol*, 2019). Nanoparticles (NPs)-mediated delivery has been shown to exhibit beneficial effects (Huang et al., *Oncotarget*, 2017). I think there are many opportunities for technologies that build upon these methods and refine them, e.g. by optimizing the frequency and amplitude of ultrasound stimulation, or the shape and coating of NPs. Computational analysis and modeling techniques can further benefit this line of research.

For cryonics, the challenge of opening up the BBB is to some extent less severe, as it is not necessary for all the cells to remain uninjured. Certain types of damage are likely to be acceptable, as long as the previously discussed information-theoretic death is not imminent. This means that certain methods that cannot be considered in clinical practice due to safety concerns, could still be employed in cryonics. For example, a major disruption of the BBB with very high frequencies of ultrasound might enable cryoprotectants to take effect during the cryopreservation process without compromising the integrity of the patient's identity. ■

Computational Neuroscience and Cryonics:
Strangers that are Just Friends Waiting to Happen

By Roman Bauer

What is Computational Neuroscience?

The human brain contains about 86 billion neurons and 10^{15} connections between neurons. No human can grasp such complexity. Computational methods are required to analyze and make sense of our observations of the brain. This is exactly the goal of computational neuroscience (CN), an interdisciplinary field looking to discover the secrets of the nervous system and how it processes information. We look at this at every scale, ranging from individual molecules to neurons and entire brain regions. CN draws on theories, skills, and models involving neuroscience, mathematics, physics, and computer science, to name just a few.

CN can be traced back to French neuroscientist Louis Lapicque, who published the first mathematical model of neuronal firing in 1907 (Lapicque 1907). It would be more than three decades until networks of neurons were modeled by pioneers such as Warren McCulloch, Walter Pitts, and Frank Rosenblatt (McCulloch and Pitts 1943; Rosenblatt 1960). These models, however, were not yet advanced enough to computationally outperform alternative existing techniques.

Advances in computer hardware and software revolutionized CN. With greater computing power, we were suddenly able to test many hypotheses and take into account a wide range of data from lab experiments. New computational theories captured essential features of the brain, and could produce important functions such as pattern recognition and associative memory (Fukushima 1988). CN also made notable contributions back to its mother discipline, neuroscience, by formalizing new concepts to explain a wide range of experimental data (Fig. 1), such as that of the function of neuronal circuits in the cortex (Douglas et al. 1989).

CN has come a long way from the simplistic models in its infancy. While we are still far from truly understanding the workings of the brain, the field of CN can boast important achievements. For instance, CN inspired powerful machine learning and AI algorithms that transformed modern industry and society, including speech and object recognition. Notably, the ideas and methods of CN also contributed to other research fields. For example, artificial recurrent neural network models are used in disease diagnosis, IT security, financial modeling, etc. These innovations are just the beginning and more insights into the workings of the brain will further advance many technologies.

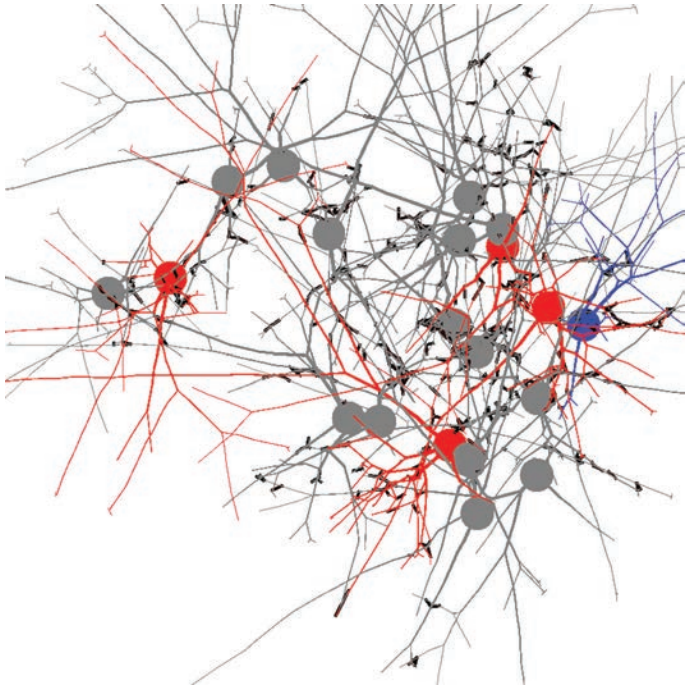

Figure 1: A computer model of a neuronal network which was "grown" based on biologically plausible developmental rules. Neurons are coloured in gray, some in red for clearer visualization of the morphologies (Bauer et al. 2014).

Current applications of CN relevant to cryobiology and cryonics

Neurobiology, cryobiology, and cryonics share many similarities. For instance, they study complex biological systems that are difficult to access and measure. In the case of neurobiology, technological, practical, and ethical challenges render it difficult to obtain detailed information on the many processes occurring in the brain. For example, the temporal resolution of fMRI is around three seconds. This is much longer than the duration of individual neuronal spikes, which take milliseconds. In the case of cryobiology and cryonics, it is equally problematic to track all the changes occurring during cooling, preservation, and thawing/devitrification. Indeed, information on where ice crystals form,

how large they are and how they perturb molecules is extremely difficult to obtain. To address these challenges, theoretical and, in particular, computational models offer appropriate toolsets to create, test, and refine hypotheses on biological dynamics.

Clearly, computational analyses and models benefited neuroscience. Arguably, cryonics-related applications could also be improved based on similar techniques.

The brain as common ground

Even though neuroscience and cryonics have different aims, they have much in common. The brain plays a key role in these subjects. In neuroscience, gaining insights on the brain is the main research objective. In cryonics, the main goal is to preserve the brain. Indeed, modern neuroscience and cryonics operate under the well-established working hypothesis that the brain defines and produces the identity of a person or animal. In other words, the brain is the seat of our personalities, memories, goals, feelings, and consciousness. For cryonic suspension to succeed, it is essential that the information defining a patient's identity is not destroyed during the cryopreservation process and can be reproduced afterwards.

One important question that arises is how the identity of a person is encoded in the brain. Even though CN offers important insights on this question, it does not (yet) have a good understanding of the "neural code." The main reason for this difficulty is the extremely complex relationship between biological neural networks and their function: the dynamics of even very simple biological neural networks can become highly complex. For instance, we know all the connections among the 302 neurons of the central nervous system of the roundworm *C. elegans* (i.e. their connectome); however, we don't understand how these neurons co-operate and give rise to behavior. To this day, there is no computer model on the planet that can predict the behavior or neuronal activity of the *C. elegans*!

Why is neural function so complex? The function of biological neural tissues is shaped by many networks of interactions. While in the early days of neuroscience it was commonly assumed that neural function is mainly defined by the synapses (i.e. connections between neurons), we now know that this is not true. The issue is much more complex: neurons have cellular properties that affect their function. The number and distribution of neuronal receptors, gene expression, and methylation, the morphologies of dendrites, spines, and boutons, and the interaction of neurons with glial cells and the vasculature all play roles in the formation and integrity of our memories (Trettenbrein 2016), hence our identity. We are still trying to understand how strongly these individual components are indeed involved. Nevertheless, any cryopreservation method will, to some extent, cause injury. It is crucial to find out how the negative impact of such injury on the cryonics patient can be avoided or mitigated. CN can play a major role in this task.

Analyzing and modeling the brain

Now is a particularly exciting time to conduct computational research involving the brain. Modern methods can reliably and accurately generate various types of neural data. Along those lines, many computer-assisted methodologies for neural tissue analysis and modeling have been developed in CN. We now have tools available to observe neurons in great detail, and to even manipulate the nervous system. There is no reason why these could or should not be employed in cryonics.

In the '90s, a sub-discipline of CN called "neuroinformatics" emerged, where the focus is on the collection, storage, and analysis of neural data. It has given rise to a rich repertoire of powerful toolsets, comprising a large user community from different fields. Well-documented software for the analysis of MRI, fMRI, DTI, CT, multielectrode arrays, PET, and EEG data can be easily obtained and employed for various purposes. Moreover, recent tools in CN have become so advanced that they can automate the acquisition of information. Algorithms can automate and speed up the reconstruction of large tissue volumes. These advanced technologies have allowed us to map the neural connectivity in parts of the *Drosophila* and mouse central nervous systems. Such tools can also help validate the integrity of neural tissues after cryopreservation, and so could also be leveraged for cryonics research. These would enable researchers to inspect the brains of cryopreserved patients in a non-invasive, automated, quantifiable, and objective manner. Despite the many challenges, there is good reason to be enthusiastic about neuroscientific and cryonic research involving the brain.

In general, CN is relevant for the following cryonics subjects:

1. Analysis of the brain

   CN researchers employ modern software to analyze and quantify neural data. Software tools can extract information on anatomical connections between brain regions, reconstruct the morphology of an individual brain, or analyze the activity of neurons recorded with multi-electrode arrays. This has many applications in cryonics, where information on brain viability is of major interest.

2. Modeling of cryopreservation

   CN has produced a plethora of models at different scales. Depending on the research question, a model characterizes ion flux across the membrane of a single neuron, the modulation of a synapse, individual spikes of neurons, firing rates of entire populations of neurons or brain regions, or even the behavior of an organism. These models were created not just to simplify but to improve our understanding of the essential dynamics taking place at these given levels. They also help test hypotheses and resolve conflicting theories.

3. Revival

Last but not least, CN tools can help guide the development of procedures that will be necessary after preservation. There are currently many open questions in cryonics that would benefit from being addressed by quantitative and computational methodologies. These relate to:

Repair and regeneration

Regardless of the technique employed, current preservation procedures have significant impact on the integrity of the preserved brains. It is still unclear what type of damage and how much of it is tolerable. To answer this question, it is crucial to understand the neural code. In other words, how do neurons operate and function together?

It might not be necessary to understand the workings of the brain to be able to preserve it. Nevertheless, such knowledge will be essential to dealing with the problem of brain injury. One possibility will be the repair of injured parts, i.e. by stimulating re-growth. This field of neural repair mechanisms is an active field, particularly in the context of spinal cord injury (Courtine and Sofroniew 2019).

Future technologies may even allow new cells and tissue to regenerate rather than repair the brain. In other words, parts of the tissue would be re-grown in such a way that they are integrated into existing parts of the brain. Computational modeling will also be helpful here to determine what behaviors the regenerating neural stem cells must be able to express. I took some initial steps in this direction in my doctoral dissertation (Fig. 2).

Transformation of consciousness

Another crucial question relates to the nature of consciousness: what are the conditions that enable us to have thoughts, to be aware, and to feel emotions? How does the brain produce a sentient being that remembers the past and has hopes for the future? Thousands of years have passed without a satisfactory answer to this mystery.

The possibility remains that the preservation process inflicts such injury to the brain that it becomes unrepairable. Fortunately, this does not necessarily mean that a person is irrevocably dead and that his/her consciousness is lost. In many cases, information-theoretic death will not yet have occurred. If the most crucial information that constitutes the person's identity is still present in the brain, then there will be ways to recover this identity and consciousness. In this case, we need to know which elements are crucial for consciousness, and which ones play mainly supportive roles.

In principle, reconstitution of a diseased person's brain could occur by transforming the functional architecture of the brain into an entity that is very different from the human brain. This target platform could be composed of biological tissue, and/or comprise non-cellular or non-carbon-based synthetic material. It would be pure speculation at this point to discuss how this should be ideally accomplished. Nevertheless, CN is also very relevant here: such transformation of consciousness will require knowledge of which aspects of the nervous tissues are required to produce a coherent identity and consciousness. It is necessary to understand how a given person's brain encodes its consciousness.

There are existing steps towards a comprehensive, computational framework of consciousness. Along those lines, the integrated information theory (IIT) was proposed as a theoretical framework that attempts to link consciousness with its physical substrate (Oizumi et al. 2014; Tononi et al. 2016). IIT has interesting implications for cryonics. For instance, according to IIT, if one were to transform all information from someone's brain into a feed-forward architecture (rather than a feed-back architecture),

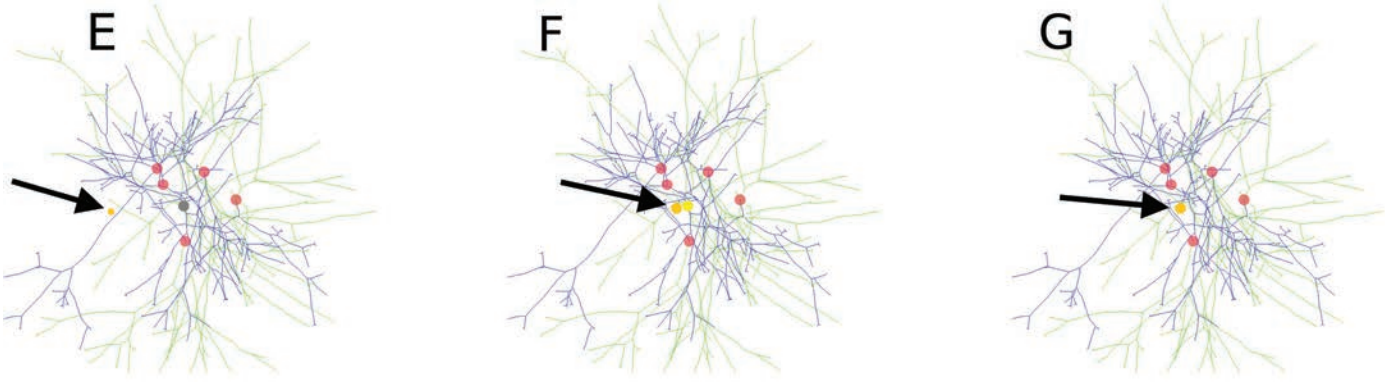

Figure 2: Simulation of the replacement of an injured cell by an engineered cell (orange cell, black arrow) that recognizes the injured cell and replaces it by integrating into an existing neuronal network. The injured cell is colored gray before replacement, and yellow afterwards. This type of simulation could help create models of neural tissue repair and regeneration. Picture adapted from (Bauer 2013).

it would not allow for the existence of consciousness. It is outside the scope of this article to discuss this theory and its implications in cryonics in more detail.

Applications of CN that can benefit cryonics research now

The tools available in CN can be employed to cross-fertilize cryonics in many respects in the future, but this begs the question: what CN applications can make a difference *now*? I believe that cryonics can indeed benefit from currently available technologies in CN, and highlight the following three applications:

Viability assessment of cryopreserved neural tissue

CN often involves the analysis of neural tissue from various measurements, e.g. images or electrical recordings. Along those lines, in the study (Peraza et al. 2019) we created an algorithm that infers the presence of Alzheimer's Disease from brain images. Similar algorithms are also very relevant for cryonics research, since these could be employed to measure the success of cryonics procedures.

Validation of past studies on neural tissue cryopreservation were usually performed on a rather superficial level. For instance, the study (Suda et al. 1974) presents measurements of EEG activity in a cat brain that was cryopreserved for over seven years. This observation is indeed fascinating, but many questions regarding the integrity of the brain remain unanswered, such as those centered around the communication between brain regions or the tissue viability. Similarly, the study (Pichugin et al. 2006) provides very encouraging evidence on the viability of rat brain tissue slices after cryopreservation. However, the electrical activity is analyzed by the rather basic measurement of potassium and sodium concentrations. Current analysis techniques in CN could provide a much more comprehensive picture of the functional state of nervous tissues. This means that they could accelerate the process of validating cryonic preservation procedures. On my team, we currently investigate the cryopreservation of retinal tissue, which is a neural tissue.

CN toolsets could support monitoring and quality checks before, during, and after cryonic preservation. These inspection methods will ideally be non-invasive, automated, and intelligent. For instance, volumetric monitoring of temperature, oxygen, cryoprotective agents, and electrical activity within the brain (Norton et al. 2017) can inform the ongoing preservation process, and trigger adjustments if necessary. The CN field has developed sophisticated methods that can remove movement artefacts, maximize spatial and temporal resolution, and automatically calculate statistical parameters. Such monitoring is additionally important because individuals differ in many properties of the brain, e.g. the shape, size, and folding patterns (Smith et al. 2019). Adaptive and personalized preservation procedures will further improve brain viability after resuscitation.

Optimization of the cryopreservation procedure

Quantitatively guided experimental procedures to maximize neural tissue quality after preservation do not (yet) exist. As evidenced by CN, the toolsets exist to model neural tissues at many different levels. Indeed, while most other biological disciplines have significantly benefited from technological advances in computing (e.g. genetics and medicine), the usage of computational tools in cryopreservation (what one might call "computational cryopreservation") has not advanced much since the 1970s. More recently however, innovative mathematical and numerical approaches to optimize the cryopreservation of cells have been created (Davidson et al. 2014), and I expect this field at the intersection of computer science and cryopreservation to expand rapidly in the near future. Needless to say, such techniques can also benefit the cryonics field.

Open and collaborative research

As explained earlier, neuroinformatics is a subfield of CN that deals with the storage, organization, and analysis of neuroscientific data. Neuroinformatics has given rise to a number of initiatives that facilitate and promote collaboration across labs. For instance, there exist large public databases such as the NeuroMorpho database or the Allen Brain Atlas that serve as a source of highly detailed experimental data. Along those lines, in the BioDynaMo project (www.biodynamo.org) that I lead we created a software framework that allows one to simulate biological tissues (Bauer et al. 2017), including neural tissues (Fig. 3). We are currently working on extending BioDynaMo to also model and simulate cryopreservation of cells and tissues. This software package will help devise cryopreservation protocols *in-silico* and reduce many costly and time-consuming experiments to determine protocol parameters.

From a more general perspective, CN has given rise to a number of large-scale projects. Very positive examples such as the Allen Institute for Brain Sciences or the BRAIN Initiative demonstrate that it is possible to create such interdisciplinary projects that grow and benefit from the expertise of all partners.

Clearly, the provision of a free and publicly accessible platform where cryopreservation and cryonics-relevant data are made available would facilitate research and exchange of ideas. Well-organized and ideally unified information on cryopreservation parameters (e.g. type and concentration of cryoprotective agents, cryopreservation methodology, cooling rate, etc.) and the final quantified viability results would contribute to validating and improving current practice in cryonics. This would facilitate a well-organized approach to assess, measure, and compare cryonic preservation procedures.

Overall, neuroscience, cryobiology and cryonics share important features, and there is a lot of untouched potential in the cross-fertilization of currently available ideas and methods. To this

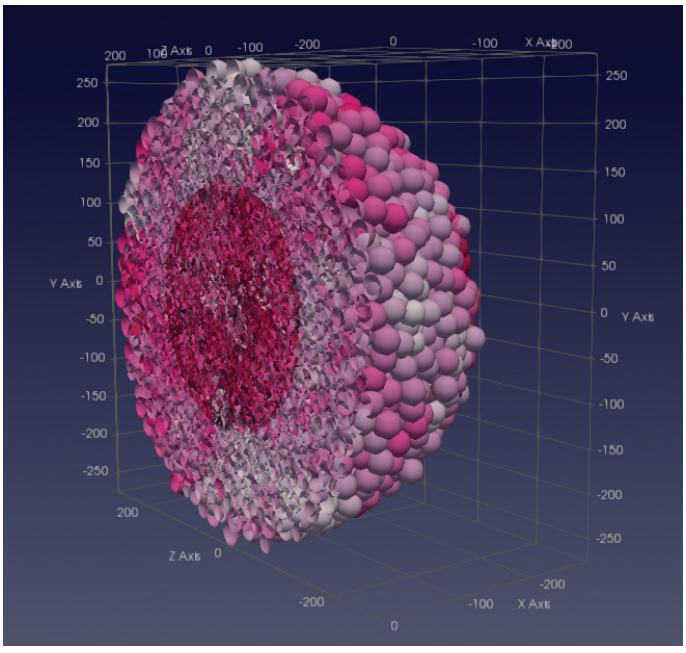

Figure 3: Simulation of a spheroid of cells, grown using BioDynaMo. Large cellular and neuronal systems can be simulated in great detail.

end, researchers will need the support and resources to work beyond the boundaries of their disciplines. It remains to be explored how the predominantly academic realm of CN can be better linked with the more application-focused cryobiology and cryonics fields. A well-organized funding structure is a key condition to realizing such interdisciplinary efforts. The current lack of significant interaction between these fields demonstrates the need for initiatives involving key players from companies, academia, charities, and research funders. If such collaboration can be accomplished fruitfully, it will benefit cryonics and society as a whole, with applications in neurology, emergency medicine or organ preservation. ■

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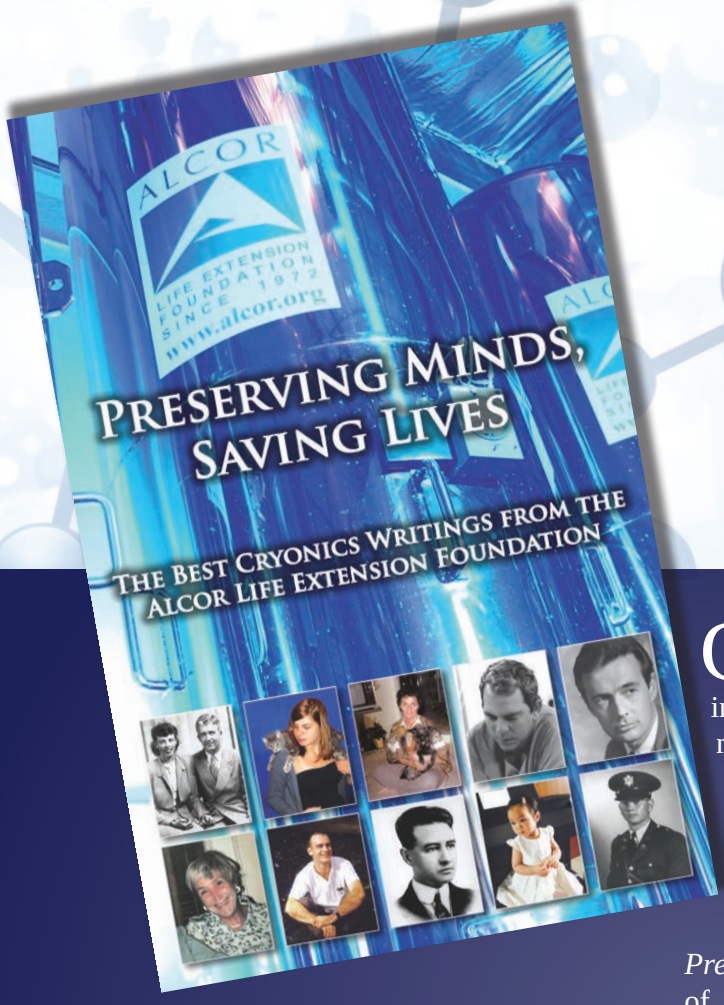

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Pro-Death Philosophy in *Star Trek Picard*

By David Brandt-Erichsen

Most filmed science fiction is really "anti-science" fiction.

For the most part, a shining exception has been *Star Trek*, which for decades has been consistently pro-science, pro-technology, and pro-space, and consistently depicting a future for humanity improving over time through science and technology and moving into space.

It hasn't been perfect in that regard, of course. *Star Trek* basically ignored the idea that advancing technology would extend human life spans. But I can forgive them for that (provided they are at least neutral on the subject) because if all the characters lived for a thousand years, and the implications of advanced nanotechnology and transhumanism were really explored, it would be hard to come up with stories that the masses could relate to.

Another imperfection was the negative (and very strange) attitude toward cryonics in the one episode that dealt with the subject – the last episode of the first season of *Star Trek: The Next Generation* (Episode 26, "The Neutral Zone").

In this episode, Captain Picard is away when the Enterprise finds a derelict 20th Century spacecraft containing three cryopreserved humans. Commander Data brings them on board and Dr. Crusher revives them (each of them had died from a "minor medical problem easily cured" now but terminal when they were frozen). Dr. Crusher stated "They were frozen *after* they died [tone of surprise]. It was a fad in the late 20th Century. People feared dying. It terrified them."

When Picard returned he was distracted by a dangerous situation with the alien Romulans and did not want to be bothered with these revived people, and he was annoyed with Data for bringing them on board. "But Data, they were already dead. What more could have happened to them?"

Data reiterated that "cryonics was never more than a fad" and Picard stated in a tone of disdain: "In this case it apparently worked. Well, they are alive now. We'll have to treat them as living human beings."

Later, there was an exchange between Picard and Ralph, one of the revived persons who had been portrayed as an arrogant financier who demanded a copy of the *Wall Street Journal* so he could see how much his stocks had appreciated over 400 years. Picard had arranged for the three to be taken back to Earth.

> Ralph: "But then what will happen to us? There is no trace of my money. My office is gone. What will I do? How will I live?"
>
> Picard: "This is the 24th Century. Material needs no longer exist.
>
> Ralph: "Then what's the challenge?"
>
> Picard: "The challenge is to improve yourself, to enrich yourself. Enjoy it!"

It is one thing to ignore that technology will extend life spans. It is another thing to misunderstand and disparage cryonics. But it is yet another thing to be actively in favor of death and to promote death as a good thing. That is actually the philosophy promoted in the final episode (Episode 10) of the first season of *Star Trek Picard*.

In this episode, Data is present as a conscious reconstruction within a simulation. He speaks to Picard: "I want you to terminate my consciousness. I want to live, however briefly, knowing that my life is finite. Mortality gives meaning to life. Peace, love, friendship – these are precious because we know they cannot endure. A butterfly that lives forever is really not a butterfly at all."

At this point my wife, who was not paying attention to the show, was startled when I suddenly yelled out "What bullshit!" I'm sure Spock would say "Illogical!"

And then Picard, who had just been cured of a fatal heart condition, said, frowning, "You didn't make me immortal [did you]?" Like that would be really awful, wouldn't it? Do Picard and the writers really hate life that much? Picard goes on to say he wouldn't mind another 10 years, or even 20.

I am reminded of the 1974 movie *Zardoz*, where a future group of immortal humans, whose needs are all taken care of, are so bored that all they want to do is die (and they can't). I think that movie was one of the biggest piles of garbage ever put on film.

Other than the garbage-philosophy anti-life anti-science ending, *Star Trek Picard* was a beautiful production and a well-done story. But my image of *Star Trek* being a shining example of pro-science fiction has been shattered. ■

Mathematics and Modeling in Cryonics: Some Historical Highlights

By R. Michael Perry and Aschwin de Wolf

Introduction

Robert Ettinger, in *The Prospect of Immortality*, his 1964 classic that largely started the cryonics movement [34], speaks of "the solid gold computer" which is to herald a "second industrial revolution." This in turn will rest "on the replacement of human brains by machines." Marvin Minsky, one of the founders of artificial intelligence (and a reported cryonics patient [23,37]) is among those quoted in support: "I believe … that we are on the threshold of an era that will be strongly influenced, and quite possibly dominated, by intelligent problem-solving machines."

Computers are, of course, intimately associated with mathematics, for what is a computer but a machine for the automated doing of mathematics and mathematical modeling in their many, varied forms, in application to problems of interest? This report will not directly concern the use of computers in cryonics, but the underlying mathematical initiatives that have occurred over the years. First came theoretical work, then practical implementation, again using computers. Cryonics has been, of course, a small movement sponsored by private interests. It is not the place where you expect to find armies of scientists and mathematicians, armed with the latest computing technology, applying algorithms on a massive scale and also developing new ones at breakneck pace. That said, the extent of mathematical investigation and application in cryonics is rather surprising, a bit more than you'd expect from the labeling of "pseudoscience" that those outside the field often attach to the practice [16].

Here we look at several efforts over the years to address cryonics problems mathematically, using layman's (mostly non-mathematical) language. We start with pioneering work of Art Quaife with his remarkable modeling in the early 1970s of perfusion systems, with a follow-up in the 1980s. We summarize Hugh Hixon's work using the Arrhenius equation to gauge the adequacy of cryopreservation temperatures. An effort of the author at modeling perfusion systems is then recounted, with use on an Alcor case. Next is a different problem: estimating the total post-pronouncement ischemic exposure of cryonics patients, until they are cooled sufficiently that further ischemic challenge is effectively halted. Some preliminary work by the author is summarized, followed with a more thorough if unpublished treatment by Steve Harris. Next is the work of Greg Fahy and others at 21st Century Medicine to predict cryoprotectant toxicity and thus shorten the labor of finding better cryoprotectants. A different problem then covered is to estimate the expected frequency of heavy caseloads, given that cases essentially occur randomly. Next is the work of Robert Freitas to address the problem of long-term organizational stability through econometric modeling. Our coverage concludes with some work by Aschwin de Wolf and others, which focuses on ischemic damage in neural tissue, including an algorithm implemented by Michael Maire to characterize ischemic damage based on machine learning.

Art Quaife's Early Perfusion Modeling Paper

Fred and Linda chamberlain, who founded Alcor in 1972, also established a sister organization, Manrise Corporation, which would deal with technical matters relating to cryonics procedures. Its journal, *Manrise Technical Review* (MTR), ran through seven issues 1971-73, crammed indeed with technical material, both theoretical and practical, on procedures for cryopreservation, as well as a detailed report on an actual case. Coming so early in cryonics history (within 10 years of the first freezings [31]) the journal stands as a remarkable tribute to the tenacity and seriousness with which the idea of cryonics was being pursued by some, as well as the high level of competence of some of those pursuing. Art Quaife's paper, "Mathematical Models of Perfusion Processes" appeared in the April-June 1972 MTR [4] and runs for dozens of finely printed, equation-heavy pages. It is a tour de force of mathematical insight into perfusion and the closely related heat-exchange problem that should still be of interest to the mathematically inclined. The abstract notes the pioneering nature of the work, then briefly summarizes the content. "Solid state hypothermia" (SSH) refers to the state of being at cryogenic temperature (usually liquid nitrogen temperature):

> Very little empirical data is available concerning the actual efficacy of alternate procedures for freezing and thawing whole mammals to[/from] the temperature of liquid nitrogen. In order to currently formulate recommended procedures for the induction of solid state hypothermia in humans, it is thus necessary to rely partly upon extrapolation from the results of experiments treating the freezing of cells and organs,

> and to an even greater extent upon theoretical analysis of the effects of alternate procedures. In this paper, we formulate mathematical models of many of the processes that take place during the induction of SSH in humans. Primary focus of the treatment is to analyze the rate of removal of heat from the body, and the rate of buildup of cryoprotectant in the body cells during perfusion. Specific formulas are also developed giving the quantity of cryoprotectant and the length of time required to accomplish perfusion, the cooling profile to be followed, and many others. The conclusions of the theory are used to formulate a specific recommendation as to a best current perfusion procedure. In many cases, the unknown values of the body parameters appearing in the equations require that approximating assumptions be made in order to achieve numerical results. It is expected that these results will be refined considerably when further information becomes available concerning the values of these parameters.

Some additional detail is added in the short Introduction:

> As currently conceived, the induction of solid state hypothermia (SSH) in the human body takes place in three phases. During Phase I, the blood is replaced by a suitable balanced salt solution and the body temperature is lowered to 0°C as rapidly as possible. In Phase II, a concentration of cryoprotectant [assumed in the paper to be dimethyl sulfoxide (DMSO)] is built up in the body water, while continuing to lower the body temperature in such a way as to remain in liquid state. Phase III begins when the desired terminal concentration is achieved; the body is then lowered to liquid nitrogen temperature where it is stored in solid state. During the first two phases, systems will be required to recirculate chilled perfusate through the body. This paper treats the mathematics of such systems.

A preliminary report on this work appeared in *The Outlook* (April 1972), before the publication in MTR [6]:

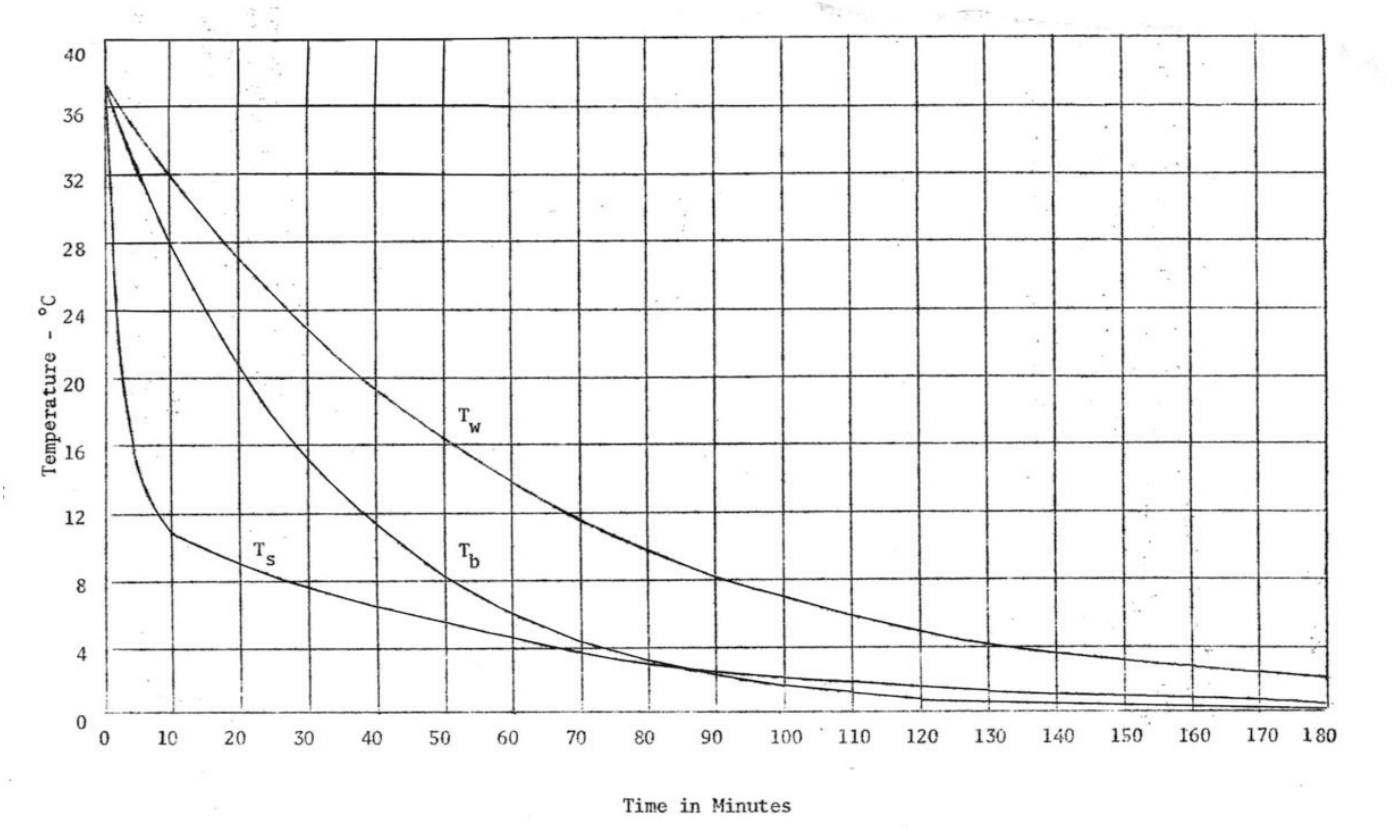
> …Mr. Quaife has gone well beyond any known previous work in applying detailed analysis to the problems involved, including the major questions of cooling rates and control of concentration of perfusate and its ingredients, and the related questions of temperature gradients, concentration gradients, minimizing of times and costs, etc.
>
> The degree of sophistication, both biological and mathematical, is considerable. Differences between organs and tissues are considered, and formulation of some problems leads to differential equations to be solved by the method of the Laplace transform. Nevertheless, it is hoped that as many as possible will read the paper, get what they can out of it, and feed back any useful comments. A revised draft will be published in an early issue of *Manrise Technical Review*.

The "revised draft" is, in fact, the paper under consideration here. The "three phases" Art Quaife refers to in the cryopreservation process ("induction of solid state hypothermia") are now called body washout (Phase I), cryoprotective perfusion (Phase II) and cryogenic cooldown (Phase III). Phases I and II involve perfusion of substances (solutions) into the body and Phase III takes the patient from approximately water ice temperature to the cryogenic range, typically the temperature of liquid nitrogen. The paper mainly concerns these first two phases, where substances are being perfused into the body and (initially) blood and body fluids are replaced. The perfusion makes use of the body's vascular system, otherwise used when the heart circulates blood. The performance of the vascular system is highly tissue-dependent, as Art explains:

> In normal circulation, there exists a wide variation in the percentage of the cardiac output delivered to different tissues. For example, on a per gram of tissue basis, the kidneys receive over 150 times as much blood as do the skeletal muscles. We can approximate the normal blood flow by dividing the body into two parts: (a) Strongly circulated tissue, consisting of the brain, heart muscle, kidneys, and organs of the hepatic-portal circulation (mainly the liver), and (b) Weakly circulated tissue, consisting of the remainder of the body.

The strongly circulated tissue makes up a much smaller volume and mass than the weakly circulated, yet the total circulation through the strongly circulated is several times greater. A cited reference estimates that only 7.3% of the body weight of an adult human male (63 kg) is strongly circulated, at 3.76 liters/min. (resting state of cardiac output, as are the other estimates here). The balance is weakly circulated, at 1.64 liters/min., for a total circulation of 5.40 liters/min. For the case of perfusion of a postmortem body for cryonics purposes a figure of 4 liters/min. total circulation is assumed, with the other values scaled proportionally (2.79 and 1.21 liters/min. respectively). In this way we obtain the "partitioned model" of the body comprising the two different flow rates in the different, nonoverlapping tissue components. Also considered is the "uniform model" in which the circulation flow rate is assumed to be the same throughout the body, and still the same overall (4 liters/min.).

As noted above, in Phase I perfusion, blood and body fluids are replaced with "base perfusate" which sets the stage for Phase II, cryoprotection. An additional effect in Phase I is to cool the body, typically from near normothermic temperature (37°C) to near the ice point (0°C). The chart below ("figure 4" in the paper) shows predicted effects (calculated temperature curves) if chilled perfusate at 0°C is circulated through tissue starting at a temperature of 37°. Shown are the three cases of (1) uniform



Predicted cooling curves for a 63 kg. man, with perfusion flow of 4 liters/minute and heat exchanger efficiency H = .4. T_s and T_w are from the partitioned body model, while T_b is from the uniform body approximation. Due to the limitations mentioned in the text, the actual rates of cooling of T_s and T_w probably lie closer to T_b than do the predicted curves.

body model (T_b), (2) strongly circulated portion (T_s), and (3) weakly circulated portion (T_w). The caption is taken from the paper, where it is noted that, due to various uncertainties, the true values may differ significantly from predictions.

Predicted cooling curves for a 63 kg. man, with perfusion flow of 4 liters/minute and heat exchanger efficiency H = .4. Ts and Tw are from the partitioned body model, while Tb is from the uniform body approximation. Due to the limitations mentioned in the text, the actual rates of cooling of Ts and Tw probably lie closer to Tb than do the predicted curves.

There is much more to the paper. Not covered here, for instance, are detailed recommendations of perfusion circuit designs and suggested flow rates during the different phases of the cryoprotection, and how to determine them. Some further, if still highly abbreviated, idea of the scope and depth of the work can be gathered from the titles of the nine main sections: (1) Limitations of the Analysis; (2) Macrocirculation of the Blood; (3) Microcirculation of the Blood; Diffusion and Heat Conduction; (4) Black Box Description of Heat Exchangers and of the Body as a Medium for DMSO Diffusion; (5) Cooling the Body; (6) Phase I Perfusion; (7) Cryoprotection; (8) Freezing Point of DMSO-Water Solutions; (9) Phase II Perfusion.

Cryoprotectant mixtures have changed since the date of this study, when DMSO (dimethyl sulfoxide) was the main ingredient [12,13,22], yet the basic approach and mathematical analysis remain valid and worthy of study.

Second Art Quaife Paper

Fast-forwarding a few years, a second Quaife paper appeared, "Heat Flow in the Cryonic Suspension of Humans: Survey of the General Theory" (*Cryonics*, September 1985) [6]. The problem of heat flow in solids has been extensively studied in physics, and Quaife offers some textbook equations that describe the general problem before proceeding to some simplified versions of the problem that approximate conditions in human cryopreservation. The abstract offers further details:

> Procedures used in the successful freezing and thawing of diverse human cells and tissues are known to be quite sensitive to the cooling and thawing rates employed. Thus it is important to control the temperature descent during cryonic suspension of the whole human body. The paper surveys the general theory of macroscopic heat flow as it occurs during the cryonic suspension of human patients. The basic equations that govern such

heat flow are presented, then converted to dimensionless terms, and their solutions given in geometries that approximate the human torso, head, and other regions of the body. The solutions are more widely applicable to the freezing of tissues and organs.

The article, then, generalizes the results of the earlier paper, then specializes the treatment to approximate cases of interest in cryonics. It is divided into thirteen main sections as follows: (1) Nomenclature; (2) Equations Governing Heat Conduction; (3) Dimensionless Variables; (4) Initial and Boundary Conditions; (5) Dimensionless Heat Flow Equations; (6) Global Reformulation of Equations; (7) Useful Mathematical Functions; (8) Basic Solution: Heat Flow in One Dimension; (9) Heat flow in a Semi-Infinite Solid; (10) Heat Flow from a Highly Insulated Solid; (11) Heat Flow from a Sphere; (12) Heat Flow from a Cylinder; (13) Analogy between Heat Flow and Diffusion.

The brief Introduction, quoted here, informs the reader about cryonics then notes how the present work extends and generalizes work completed earlier (above). Calculations are promised for later articles, though not published as far as we are aware:

Cryonic suspension is the freezing procedure by which human patients are preserved, after pronouncement of legal "death," in hopes of eventual restoration to life and health. The procedure attempts to preserve the basic information structures that determine the individual's identity. These include the memories and personality as encoded in the macromolecules and neuronal weave of the brain, and the genetic information stored in DNA.

The author has previously formulated a mathematical model of the heat flow and the diffusion of cryoprotectant that occurs during the first phase of this procedure, in which chilled blood substitutes and cryoprotective solutions are perfused through the vascular system. The present paper treats the general theory of heat flow, particularly at sub-zero temperatures after perfusion has ceased and the body has solidified.

The author has written a computer program that calculates most of the solutions given below, and in subsequent articles intends to present tables and graphs comparing theoretical projections with experimental data. Other problems for subsequent analysis include

HEAT FLOW IN CRYONIC SUSPENSION

HEAT FLOW		⟷	DIFFUSION	
Quantity	*Unit*		*Quantity*	*Unit*
ρcT	J/m^3		C	kg/m^3
α	m^2/s		D	m^2/s
q	$J/(m^2\ s)$		j	$kg/(m^2\ s)$
F	J/m^2		M	kg/m^2
Q	J		m	kg
$h/\rho c$	m/s		α	m/s
Equation	*Name*		*Equation*	*Name*
$q = -k\nabla T$	Fourier's law		$j = -D\nabla C$	Fick's first law
$\nabla^2 T = \frac{1}{\alpha}\frac{\partial T}{\partial t}$	Fourier's equation		$\nabla^2 C = \frac{1}{D}\frac{\partial C}{\partial t}$	Fick's second law
$k\frac{\partial T}{\partial t} = -h(T - T_\infty)$	boundary condition		$D\frac{\partial C}{\partial t} = -\alpha(C - C_\infty)$	boundary condition

Table of correspondence for heat flow versus diffusion

> change of phase, and the thermal stresses from temperature gradients within the frozen tissue.

Next, after variables are defined and terminology is established, basic equations are given: heat flow in a solid, average temperature over a volume in space representing an object such as a cryonics patient, average temperature over the surface of the volume in question, and so on. The general equations are, of course, "textbook physics" and can be found in standard references but are then specialized to address matters of interest in cryonics. Of particular interest is the treatment of heat flow in a sphere and a right circular cylinder, which approximate a human head and torso, respectively. In both cases solutions are greatly simplified over the general case, both from the simple geometry and by assuming uniform thermal conductivity which doesn't vary with temperature. (The cylinder is also assumed to be infinite in both directions, admittedly a bit of a "stretch" for a real patient, but presumably not very different inside except near endpoints.)

The work is, in fact preliminary. No calculations are offered, only formulas. That said, it is a very good start, and going further and implementing an actual modeling of a patient would be feasible but daunting and has not been attempted yet or at any rate nothing is published about such an attempt, as far as we are aware.

The paper primarily deals with heat flow, which is certainly important in cryogenic cooling, but leaves aside another problem, that of inducing cryoprotectant in desired concentration before deep cooling begins. Here, however, physics is emphatically on our side, as Quaife noted in his earlier study and reminds us here: Diffusion of a substance in a fluid medium (where mass is being transferred rather than energy in the form of heat) is mathematically equivalent to heat flow in a solid. As a parting shot Quaife in his second paper provides a table of correspondence between the two.

How Cold Is Cold Enough?

Cryonics depends on the idea that, if you store a biological sample at a low, subfreezing temperature it will be essentially unchanged for (at least) a few centuries. But how can you reliably estimate the rates of change in materials at different temperatures? And how cold do you have to be that the rates you are interested in are low enough that you can stop worrying? Biochemist Hugh Hixon, a long-time Alcor staff member still employed there today, set out to answer this question in the mid-1980s ("How Cold is Cold Enough?") [21]. His approach was to use the well-known Arrhenius equation to estimate and compare chemical reaction rates at different temperatures.

Though a good start, this approach encounters a major difficulty: in a tissue sample there are many chemical reactions going on, and their rates, which might be well-approximated individually by the Arrhenius equation, vary widely. So, for instance, a certain reaction might slow down by a factor of two when you drop the temperature a certain amount, while another might slow down by a factor of five. Hixon addresses the difficulty by selecting one important substance, catalase, and basing all conclusions on its reaction rate as a function of temperature:

> I am going to be pessimistic, and choose the fastest known biological reaction, catalase. I'm not going to get into detail, but the function of the enzyme catalase is protective. Some of the chemical reactions that your body must use have extraordinarily poisonous by-products, and the function of catalase is to destroy one of the worst of them. The value for its E [important in the Arrhenius equation] is 7,000 calories per mole-degree Kelvin. It is sufficiently fast that when it is studied, the work is often done at about dry ice temperature. My friend Mike Darwin remarks that he once did this in a crude fashion and that even at dry ice temperature things get rather busy. Another reason to use it is that it's one of the few I happen to have. E's are not normally tabulated.

With this choice, it is then possible to compare reaction rates as the temperature is lowered from body temperature (37°C) on down. And the results, overall, are reassuring, at least for the usual cryogenic storage. What takes one second to happen at body temperature would take about 25 million years at the temperature of liquid nitrogen! For temperatures warmer than liquid nitrogen the rates will be faster, but the tentative conclusion is that -115°C would slow things down enough that 100 years of storage would be equivalent to 12 hours at body temperature, at least barely acceptable. (The point is made too that below about -135°C translational molecular motion is inhibited so safe storage of almost indefinite length should be possible, irrespective of the Arrhenius equation.)

Perfusion Modeling

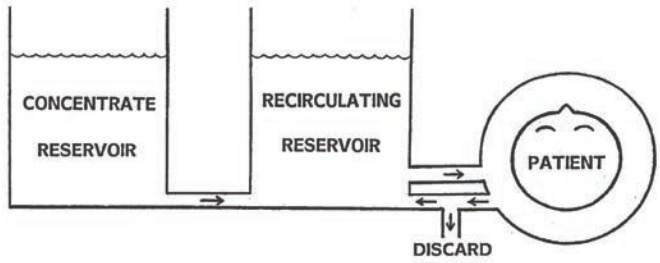
The next study was done by Perry ("Mathematical Analysis of Recirculating Perfusion Systems, with Application to Cryonic Suspension," *Cryonics*, October 1988) [24]. Less comprehensive and general than the Quaife articles, it deals mainly with cryoprotective perfusion (Quaife's Phase II), but also reports some use with an actual case, where predicted and measured values of cryoprotectant concentrations are compared. Here the patient's temperature has been lowered to near the ice point (0°C) and the base perfusate introduced in Phase I is replaced with cryoprotective agent (CPA) to protect the tissues during cooldown to cryogenic temperatures (Phase III). The conclusion of the article summarizes the main rationale and results of the study:

> Cryonic perfusion, undertaken to protect tissues from damage during the freezing process, is nonetheless

not a completely benign process. Cells and tissues can suffer damage during perfusion from (1) toxicity of cryoprotective agent(s), (2) osmotic stress, and (3) stress resulting from mechanical forces under excessive fluid pressure. For this reason the perfusion process must be carefully controlled so that CPA is introduced rapidly but without extreme concentration gradients or excessive pressure. Perfusion circuits currently in use by Alcor allow careful control of CPA buildup through control of the flow rates into and out of the patient and other fluid reservoirs involved in the perfusion. How to control the fluid flow rates to achieve an effective perfusion is a complex problem, but one that can be addressed through mathematical modeling of perfusion circuitry. It thus becomes feasible to predict with reasonable accuracy the rate of increase in CPA concentration for cryonic perfusion circuits now in use. The method shows promise in elucidating what is happening to a patient during perfusion, in comparing different perfusion protocols [based on] quantities related to cell and tissue stress, and in selecting protocols to achieve optimal perfusion under given models of stress.

In the paper, a perfusion system consisting of *n* reservoirs is modeled with arbitrary, pairwise interconnections and flow rates that are constant with time. A binary solvent-solute mixture is circulated through the reservoirs, with mixing of incoming fluids in each reservoir assumed to be instantaneous. We then wish to know the volume-for-volume (v/v) concentration of solute in each reservoir as a function of time. This in turn depends on a linear, ordinary differential equation involving flow rates into and out of the reservoir. The equation is solved numerically, using a Taylor's series approximation, and is then generalized to the case in which the flow rates vary with time.

Application of the technique to the problem of CPA perfusion during a cryopreservation is then considered. For this case we have 4 reservoirs ($n = 4$): the patient, a concentrate reservoir, a recirculating reservoir, and a discard. (See illustration.)



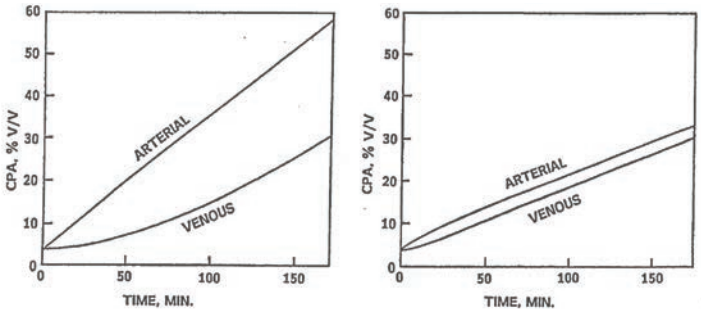
Perfusion circuit as modeled in the paper has 4 reservoirs including the patient and discard.

The direction of flow of perfusate for the different reservoirs is as follows: (1) concentrate: 1-way, to recirculating; (2) recirculating: 2-way, to/from patient; (3) patient: 2-way, to both recirculating and discard, from recirculating; (4) discard: 1-way, from patient. The two most important flow rates are (1) flow rate into patient from recirculating reservoir (f_{IN}) and (2) flow rate from patient to discard (f_D). The difference f_{IN}- f_D is the rate of flow from the patient back to the recirculating reservoir, assuming no change in volume in either the recirculating reservoir or the patient, so that f_D is also the flow rate from the concentrate to the recirculating reservoir.

In the course of perfusion, the concentration of solute in the recirculating reservoir and then the patient increases. In an actual case the perfusate is circulated in the patient's vascular system, divided into arterial (ingoing) and venous (outgoing) components. The arterial-venous or "a-v" difference in the two concentrations is a measure of osmotic stress: a greater difference signifies a greater stress, which we would like to reduce as far as possible, consistent with avoiding excessive flow rates that might cause edema. Reduction occurs by recirculating the perfusate between the patient and the recirculating reservoir as shown in the illustration. In absence of recirculation (perfusate discarded from patient, no inflow back to recirculating reservoir) we would obtain a "one pass" perfusion circuit with correspondingly larger a-v differences.

Putting it all to the test

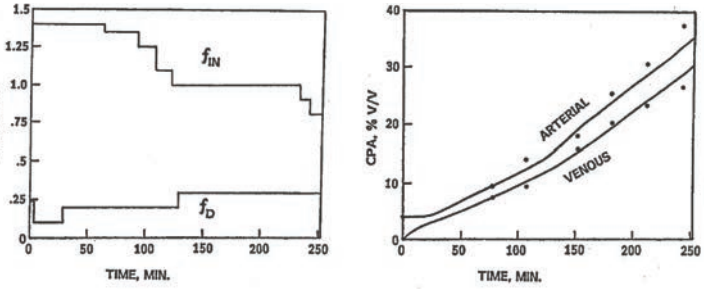
A computer program was written to predict the concentration of CPA in the patient and elsewhere as a function of time, assuming constant flow rates into and out of the patient and the reservoirs involved in perfusion. (In practice the only other case of interest that requires calculation is the recirculating reservoir.) The program was used to simulate perfusions, showing how recirculation improves the quality of perfusions by reducing a-v differences.



Simulated perfusions. Left is single-pass (no recirculation) and shows much greater a-v differences than right which uses recirculation under otherwise similar conditions.

The program was then used during a whole-body cryopreservation at Alcor (James Binkowski, starting 8 May 1988) [32,33]. Rough estimates were obtained, in advance, of time and perfusate requirements under different assumed flow rates, and actual flow rates were adjusted accordingly to reduce

anticipated osmotic stress. A more careful study was done afterward to try to reconstruct the course of CPA perfusion. Comparison of predicted and observed CPA concentrations shed light on certain physical changes, such as reduction of patient circulating volume, believed to have been caused by vascular occlusion secondary to ischemic clotting. Other programs were then written that allowed for variable flow rates, to obtain optimal perfusion profiles under certain models of cell stress. Calculations from one of these programs are shown below.



Left: flow rates for actual perfusion.
Right: observed arterial-venous CPA concentration (dots), and values calculated from model (solid curves).

A section of the paper on neuropreservation showed how this procedure would lead to a reduction in osmotic stresses over the whole-body case, under starting assumptions that seemed likely at the time. It was hoped that further programming efforts might lead to improvements including even a computer-controlled system for cryoprotection. This did not materialize, however, and use of the program was discontinued when it appeared that hands-on experience was an adequate guide for directing the course of cryoprotection. (At the time the study was carried out the CPA in use was glycerol. This use has been superseded [13], but, just as in the Quaife studies based around DMSO, the mathematics could apply to other choices.)

Another complicating factor is the effect of ischemia on flow rates and tissue equilibration. As the duration of ischemia increases, perfusion of the brain is progressively compromised due to the so called "no-reflow" phenomenon. For a given pressure (say 100 mmHg), flow rates will slow down significantly in ischemic patients, increasing perfusion times. Another adverse effect of ischemia is the development of swelling (edema) and intracranial pressure. Under such conditions a very smooth increase in cryoprotectant may not be desirable because it leads to significant water accumulation in the cells and tissues before exposure to the highest concentrations. It is also believed that a steeper increase of the cryoprotectant can be beneficial because the higher osmotic difference can recruit (edematous) water back into the circulatory system. For example, in an ischemic patient cryoprotection would not start at 0% CPA but 5% CPA. The understanding of various perfusion protocols on the ischemic patient is still rudimentary and no formal models are available yet.

Estimating Ischemic Exposure

Next we consider a problem in cryonics that is not related to perfusion or heat flow per se yet is certainly a vital concern: ischemic exposure of the patient prior to deep cooling. Historically, this problem was considered by Perry in an article in *Cryonics* (2nd Q 1996) [26] and later treated at much greater length in an unpublished (and incomplete) study by Steven B. Harris, MD [39]. Still later, Perry and de Wolf considered the problem of what cooling rate would be needed to escape ischemic injury, based mainly on Perry's earlier results [8].

What follows is adapted from the *Cryonics* article with an extension to summarize the Perry and de Wolf and Harris work. The Harris term E-HIT has been substituted for the author's original MIX ("measure of ischemic exposure") and his normalization is used. (E-HIT of 1 means 1 hour of exposure at body temperature of 37°C.)

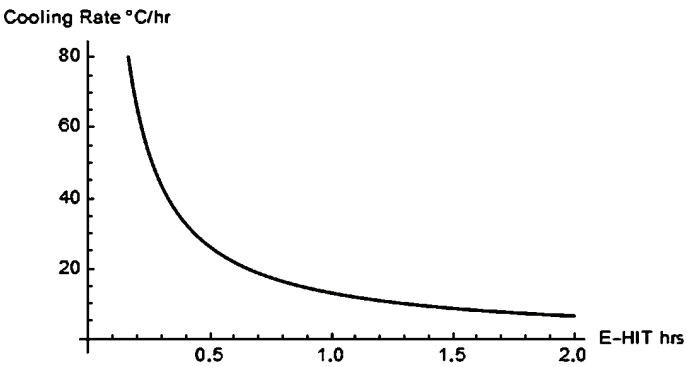
An ever-present unknown in cryonics is the quality of a cryopreservation. Until more is known, in fact, we will have no good assessment of preservation quality in terms of what we'd really like to know: how well memory and other identity-critical elements are preserved in the cryopreserved remains. Meanwhile we are interested in whatever reasonable indicators of preservation quality it may be feasible to compute, though acknowledging these are imperfect.

One such possible indicator would be an "equivalent homeothermic ischemic time" (E-HIT) intended to assess the amount of high-temperature exposure the patient experiences, mainly (not exclusively) in the early stages of cryopreservation before the temperature falls below the ice point (0°C). Basically, the E-HIT would tally up how long, in hours say, the patient has been at a given temperature, with a heavier weighting used for higher temperatures, since more damage is occurring at these temperatures. According to a rule of thumb in wide use, each decrease of 10°C is supposed to cut in half the amount of damaging activity. At least this is considered roughly accurate – though it must not be pressed very far. Here this "Q-10" rule is adopted with the understanding that it is only a starting point.

So, if we normalize our measure so that an E-HIT of 1 corresponds to one hour at body temperature (37°C), then, one hour at 27°C would be an E-HIT of only 0.5, one hour at 17° only 0.25, and so on. More generally, for a temperature T in °C the E-HIT for one hour exposure is one-half raised to the power of one tenth the difference between body temperature and T, measured in degrees C, or in mathematical notation, $(1/2)^{(37-T)/10}$, in units of hours. We also assume that the damage scales linearly with the time of exposure; two hours at a given temperature would produce twice the amount of E-HIT as one hour at the same temperature, and so on.

So far we have considered the E-HIT if the patient is maintained at a constant temperature for an interval of time, but with a variable temperature we divide the time into small subintervals of approximately constant temperature and add up the contributions of each subinterval to obtain the total E-HIT. Mathematically, temperature is now a function of time *t*, that is *T*(*t*), and we must integrate the corresponding (instantaneous) E-HIT function, $(1/2)^{(37-T(t))/10}$, between two time limits defining the start and end of the time interval where cooling occurs, to obtain the total E-HIT.

Although this can get complicated, it turns out that the E-HIT takes a particularly simple form for the case of a constant cooling rate: the E-HIT for this case is just inversely proportional to the cooling rate. The proportionality constant will depend on particulars such as the time interval, the value assumed for body temperature, exactly how much reduction in damage rate occurs per unit drop in temperature (simple variants of the Q-10 rule, more or less than a 50% reduction per 10° drop, are possible), and so on. The chart below shows the cooling rate needed to achieve a given value of E-HIT, assuming the Q-10 rule, with cooling proceeding from body temperature to 3°C, approximately where cryoprotective perfusion typically occurs. As expected, reducing the E-HIT requires a faster cooling rate, while a slow cooling rate will produce a large E-HIT because a long time will be needed to achieve the targeted drop in temperature.



Cooling rate needed to achieve a given E-HIT, for constant-rate cooling from 37°C to 3°C, assuming the Q-10 rule.

It is instructive to consider something like a typical cryopreservation under presumed good conditions to see what the total E-HIT would be. Our model cooldown will be in three stages: (1) initial cooldown from body temperature to 3° at a "typical" rate of 15°/hour; (2) pause at 3° for 4 hours (= 240 minutes, close to the case of the previous section) for cryoprotective perfusion; (3) resumption of cooldown, again at 15°/hour, from 3° down to 123.3°, the glass transition temperature of cryoprotected tissue, where we assume that further deleterious change is halted. With these assumptions the total E-HIT will be 1.34 hours, with the breakdown as follows: stage 1: 0.87; stage 2: 0.38; stage 3: 0.09. (Actually, not much is known about the real contribution to E-Hit of cooling below 0°C, but it is expected to not be much larger, and maybe much smaller, than what follows from the Q-10 rule, and small in any case for reasonable cooling rates.) We see then that most of the E-HIT comes from the initial cooldown to the start of cryoprotective perfusion. A lesser amount, order of half as much, happens during the perfusion, while a much smaller amount still is from the further cooldown to the cryogenic range.

It should be emphasized that the E-HIT as we have computed it rests on the assumption that the patient is not ischemic prior to the start of cooling, that the cooling rate is uniform as indicated, and that no assistance such as metabolic support is used to lessen the E-HIT. Another point worth making is that, of course, one would like as small an E-HIT as possible, which raises the question of how small an E-HIT might have to be to be considered insignificant and not a matter of concern. Here, however, the answer is not particularly encouraging: An E-HIT of just five minutes (0.083 h) will begin to produce brain damage, as occurs in people who undergo a delayed resuscitation after cardiac arrest. Ideally, then, we'd like our total E-HIT not to stray outside the five-minute limit; unfortunately, this is not feasible and in fact E-HITs far in excess of this limit are the best we can achieve, as suggested by the example just considered. (More specifically, it would take a speedup factor of 16.1, or a cooldown rate of 4.0°/min., plus a similar reduction by a factor of 16.1 in the perfusion time at 3°C to just 14.9 min., to achieve an E-HIT of only 5 minutes! Basically, similar results were noted by Perry and de Wolf using somewhat different assumptions about the cooling/perfusion protocol, with infeasible cooling rates needed even skipping the pause at 3° for cryoprotective perfusion.) While this exercise has shown quite dramatically that an E-HIT of 5 minutes cannot be achieved through cooling alone, cryonics organizations employ other means to protect the brain during stabilization. For example, the results in an "ideal" cryonics case are more favorable because of the use of (mechanical) cardiopulmonary support (CPS) and the administration of cerebroprotective medications. Further work needs to be done to incorporate the effect of restoring circulation to the brain in these calculations. For example, can the E-HIT number be reduced by 50% if the patient is oxygenated during CPS and blood substitution?

We note here in passing some research directed toward reducing the initial whole-body cooldown time (body temperature around 3°C), in which mathematical modeling is used. Currently the best method is still "cardiopulmonary bypass," CPB, in which the blood and body fluids are circulated through a heat-exchanger and oxygenator to maintain metabolic support while cooling occurs. Alternatives that have been tested involve circulating cold fluid through the lungs ("liquid ventilation" or "total liquid ventilation," TLV) and a variant using gas as well as liquid ("gas/liquid ventilation," GLV). A study by Steve Harris, M.D. et al. using a canine model achieved cooling rates comparable

to those of CPB with a much less invasive, GLV procedure [20], though the technique has not yet found use in cryonics. However, the study is also notable for elaborate mathematical modeling based on Harris's study of the cooling process, which we now consider.

Harris in his unpublished work recognized that the assumption of constant-rate cooling is often unrealistic. Instead what is commonly encountered is that the cooling rate itself is a decreasing exponential. That is, one has a starting temperature T_0 and a target T_1, and after waiting time w the temperature one is at, call it T_i, is half-way between T_0 and T_1, and after that same additional time (a total of $2w$) it is half-way between T_i and T_1, and so on. True, at this rate the target temperature T_1 will never be reached but the approach can be quite close after only a few multiples of w, or T_1 might actually be lower than the desired target, which in turn could be reached quickly. The assumption of exponential cooling rates leads to a more complicated, but still computationally manageable, determination of E-HIT which better tracks what is really happening. Dr. Harris in his extensive study also offers insight into reducing E-HIT by such means as metabolic support. At present, though, the work on E-HIT is still preliminary, and more work is needed to produce a trusted indicator of brain injury or stress during the cryopreservation process.

The development of a quantitative outcome measure does not need to be confined to the induction of hypothermia. CT scans can be used to develop a score corresponding to the amount of ice formation observed in the brain. These scores can be combined to create a comprehensive outcome metric for a specific cryonics case. For example, if we limit ourselves to eliminating ischemia and ice formation, a "perfect" cryonics case would be one with an E-HIT of 0 (minutes of normothermic ischemia) and no ice formation in the brain (as inferred from CT scans).

Predicting Cryoprotectant Toxicity

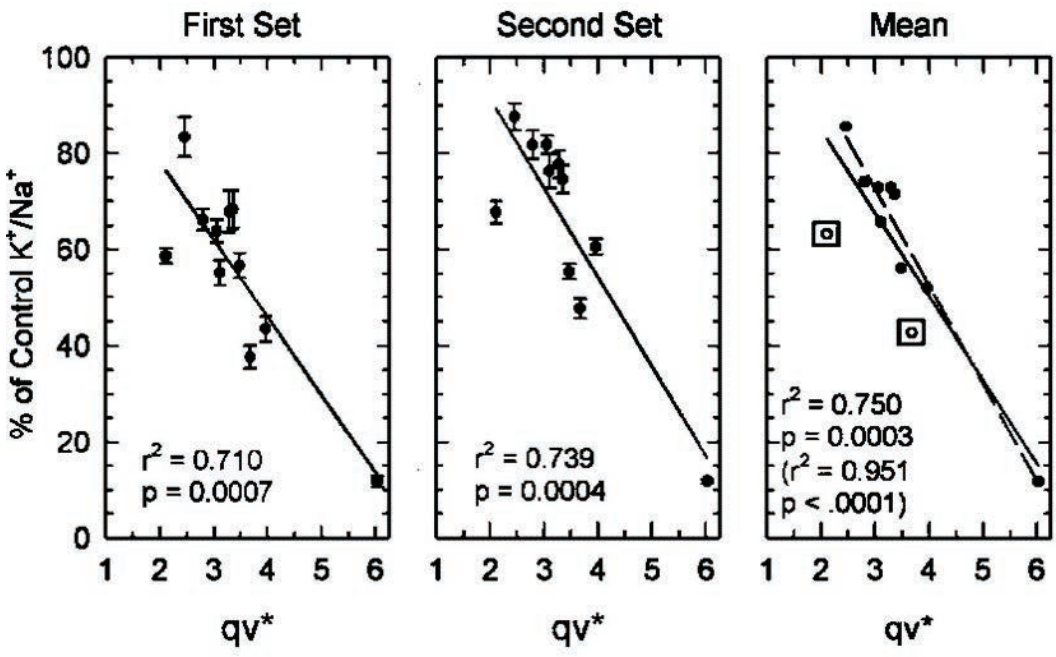
Our next example is a simple one mathematically, but in other ways rather involved: to estimate the toxicity of a cryoprotectant mixture (CM) to be used in the cryoprotective perfusion step of the cooldown process (see above). *This step is important*. With today's protocols, such as the one now in use at Alcor, the tissue on further cooling to cryogenic temperature enters a glassy or "vitrified" state in which damaging ice crystal formation does not occur and damage overall, by appearance, is greatly reduced. Unfortunately, there is a tradeoff: known CMs also are toxic. Just warm the tissue up from a vitrified state and it either does not resume function, or if it does, its function is likely to be impaired except in the case of small tissue samples such as rat neonatal intestine, blood vessels, cartilage, corneas, or mouse ovaries [38,14]. Finding a CM that has minimum toxicity but still vitrifies at realistic cooling rates is thus a priority. How does one go about doing this? Experimenting with different possible CMs is a laborious process. Is there any way to shorten the labor?

Work of cryobiologists Gregory Fahy, Brian Wowk and others at 21st Century Medicine (21CM) uncovered a method of predicting the toxicity of cryoprotective solutions to a fair accuracy based on their molecular constituents. Results are detailed in a 2004 *Cryobiology* article [17], where the authors summarize their work as follows:

> The mechanisms of toxicity of vitrifiable solutions have not been elucidated. In part for this reason, it is not presently possible to predict the toxicity of either individual cryoprotective agents or mixtures thereof, and there is a virtually unlimited number of possible mixtures to choose from in composing candidate vitrification solutions. It would therefore be of considerable practical utility to have a simple method for predicting the toxicity of a complex mixture of highly concentrated cryoprotectants from first principles.
>
> In the present contribution we show that a simple new compositional variable (qv*) can rationally account, in an organized mammalian tissue, for the toxicity of many complex cryoprotectant mixtures composed to be at total concentrations that are just sufficient to permit vitrification at slow cooling rates at both ambient and elevated pressures. This new compositional variable is proposed to reflect the strength of cryoprotectant hydration within the solution. Based on this interpretation, we were able to predict and successfully test several superior new vitrification solutions with low toxicity for mouse ova, kidney slices, whole rabbit kidneys, and other sensitive systems. These results provide substantial new support for the possibility of developing successful methods for the long-term banking of medically needed tissue and organ replacements.

"Toxicity" in turn is a term that needs visualization if one is to predict it. The visualization settled on by the 21CM researchers depends on the fact that viable mammalian cells contain high concentrations of potassium ions (K^+), versus lower concentrations of sodium ions (Na^+). Though exact proportions vary from experiment to experiment, typically there will be 5 to 7 times as many K^+ ions as Na^+ ions within healthy, untreated cells (controls) [17 p. 30, 19] which can be compared with cells to which cryoprotectants have been applied. In general, the ratio K^+/Na^+ will be lower for these other cases, the difference with controls serving as the indicator of toxicity. One can then search for cryoprotectants that minimize toxicity, that is, yield K^+/Na^+ ratios that are close to the high levels of controls.

Still there is a major problem, as the authors point out. In searching through the many possible CMs for those of low toxicity, testing each mixture individually would be infeasible. Instead the authors propose the quantity qv* which can be calculated for



Reproducibility of the correlation between K^+/Na^+ ratio and qv for 11 different vitrification solutions. The left panel recapitulates K^+/Na^+ data collected in Maryland in 1986, while the middle panel shows data collected in California in 2000. The right panel shows the average of these two data sets. The boxed points in the right panel are results that deviated from the regression line by more than 10% in both data sets. Except for these two solutions, the linear regression for the pooled results (dashed line) explains 95% of the variance of the data ($r^2 = 0.95$).*

each mixture and from which the likely toxicity can be estimated. Some results of testing are shown in the chart below, taken from the paper (caption also adapted). Eleven vitrification mixtures or VMs, which are CMs at the minimum concentration needed to vitrify under moderate cooling rates of 10°C/hour, were tested for their toxicity with results plotted against their qv* values. In most cases there was a strong correlation with qv* as shown by the resulting least-squares regression lines: A qv* of 2 or less signified a K^+/Na^+ ratio around 85% or more of that of controls (low toxicity) while a qv* around 6 signified a K^+/Na^+ ratio only 10% that of controls (high toxicity).

Calculating qv* [17,11,10,40]

To calculate qv* we look at a standard volume of a VM, say 1 liter. (The choice of standard volume is for convenience; different choices will give the same results.) This liter of solution will consist of (1) water, (2) "permeating" (or "penetrating") cryoprotectants (PCs) that are needed for vitrification but contribute to toxicity, and (3) other, nonpermeating solutes (NPs) such as carrier solution solutes or ice blockers that are usually present in modest quantity and more or less are inert as far as toxicity is concerned. (Note: by referring to the CM as a "VM" we are assuming it is at the minimum concentration needed to vitrify for a cooling rate of 10°C/hour, as noted above.) qv* is then defined as the ratio M_W/M_{PG} where M_W is the number of moles of water and M_{PG} is the "total polar moles" of the PCs.

A mole of a molecule is the weight in grams equal to its molecular mass. Hydrogen (monatomic, H) has a molecular mass of nearly 1 so 1 mole of hydrogen is about 1 gram. (The number of molecules in a mole is also a fixed quantity, Avogadro's number, about 6×10^{23}.) Water (H_2O) has a molecular mass of 18.015, so this amount in grams makes a mole of water. For a PC we must consider, in addition to how much of the substance we have in moles, the number of "polar groups" it has on its molecule. Polar groups are one of the four chemical groups: amino (NH_2), carbonyl (C=O), hydroxyl (OH), and sulfinyl (S=O) that have been identified as important in the action of PCs. Each PC will have one or more of these polar groups per molecule. So, for example, the cryoprotectant dimethyl sulfoxide (DMSO) has one sulfinyl group per molecule and no other polar groups so its polar groups number is 1. Ethylene glycol (EG) on the other hand has two hydroxyl groups per molecule and no other polar groups so its polar groups number is 2.

We then define the polar moles of a given amount of PC as the number of moles of the PC times its polar groups number. The total polar moles MPG of a VM is the sum of the polar moles of all the PCs in the mixture.

The tabulations below show calculations of qv* for two VMs, VM-1 ("vitrification mixture 1"), and M22. (VM-1 was developed by Dr. Yuri Pichugin and used by the Cryonics Institute. M22 was developed by Drs. Fahy and Wowk at 21CM and is currently used

	A	B	C	D	E	F	G	H	I
1	substance	wt., g	molar mass, g	density, g/ml	vol., ml	moles	polar groups	polar moles	qv*
2									
3	DMSO	300.0	78.13	1.100	272.7	3.840	1	3.840	
4	EG	300.0	62.07	1.113	269.5	4.833	2	9.666	
5	m-RPS-2				20.0				
6	NW				562.2			13.506	
7	W				437.8	24.302			
8	VM-1				1000.0				1.80

Calculation of qv for VM-1. VM-1 consists of an aqueous solution of the two permeating cryoprotectants dimethyl sulfoxide (DMSO) and ethylene glycol (EG) plus the nonpermeating solutes in the carrier solution m-RPS-2. Volumes of these substances that would be present in 1,000 ml of solution are added to obtain the non-water (NW) volume, which is subtracted from 1,000 to obtain the volume of water (W).*

	A	B	C	D	E	F	G	H	I
1	substance	wt., g	molar mass, g	density, g/ml	vol., ml	moles	polar groups	polar moles	qv*
2									
3	DMSO	223.1	78.13	1.100	202.8	2.855	1	2.855	
4	EG	168.4	62.07	1.113	151.3	2.713	2	5.426	
5	F	128.6	45.04	1.133	113.5	2.855	2	5.710	
6	NMF	30.0	59.07	1.011	29.7	0.508	2	1.016	
7	3MP	40.0	106.12	1.114	35.9	0.377	3	1.131	
8	PVP K-12	28.0			28.0				
9	X-1000	10.0			10.0				
10	Z-1000	20.0			20.0				
11	LM5				20.0				
12	NW-LM5	648.1			591.2			16.138	
13	W	411.9		1.000	[411.9]	22.864			
14	M22	1080.0		1.080	1000.0				1.42

Calculation of qv for M22. M22 is an aqueous solution containing the five permeating cryoprotectants dimethyl sulfoxide (DMSO), ethylene glycol (EG), formamide (F), N-methylformamide (NMF), and 3-methoxy-1,2-propanediol (3MP). In addition, it has the three nonpermeating solutes: polyvinyl pyrrolidone (PVP) K-12 and the two ice blockers X-1000, and Z-1000. Besides this are the (nonpermeating) solutes in the carrier solution LM5, here assumed to occupy the same volume as the solutes in the carrier solution m-RPS-2 of VM-1, above. The amount of water (W) is estimated from the known density of M22 rather than by assuming that volumes of all substances add on mixing. Instead, weights of the different components in a liter of solution are subtracted from the weight of the solution, in this case, 1,080 g, to obtain the weight of water (411.9 g) and thus the moles of water (row 13, col. F), which is divided by the total polar moles (row 12, col. H) to obtain qv*.*

by Alcor [7,11,12,13,15,17,18].) Both VMs consist of an aqueous solution containing PCs and one or more NPs.

VM-1, the simpler of the two, has two PCs (DMSO, EG) and one group of NPs, those present in the "carrier solution" m-RPS-2 ("modified renal perfusion solution two"), with the balance water. Volumes in milliliters (ml) of the different components are shown in column E of the table. For the PCs the volume is the weight as shown (col. B) divided by the density (col. D). The carrier solution NPs' combined volume is estimated at about 20 ml/liter [11,10]. The volumes of the PCs and NPs are added to obtain the total non-water volume (NW), which is subtracted from 1 liter = 1,000 ml to obtain the volume of water (W) in the solution, which in turn gives the weight of water in grams (again, 1 ml of water is 1 g). (Note: we assume here that volumes of different substances add on mixing, something that is not strictly true but does appear to hold to a good first approximation in cases like this one [17 p. 29]. Actually, the volumes added together will tend to be slightly less than the sum, due to the way molecules of different liquids "pack" together in solutions. This is taken into account for the more complicated case of M22, below, where it slightly increases the value of qv*.) Knowing the weight and that 1 mole of water weighs 18.015 grams gives amount of water in moles (row 7, column F), and similarly we obtain the amount of the two PCs in moles. Finally, multiplying the moles of a PC by its polar groups number (col. G) gives its polar moles (col. H). The polar moles of the PCs are added (row 6, col. H) and the total divided into the moles of water to obtain the value of qv*, in this case 1.80.

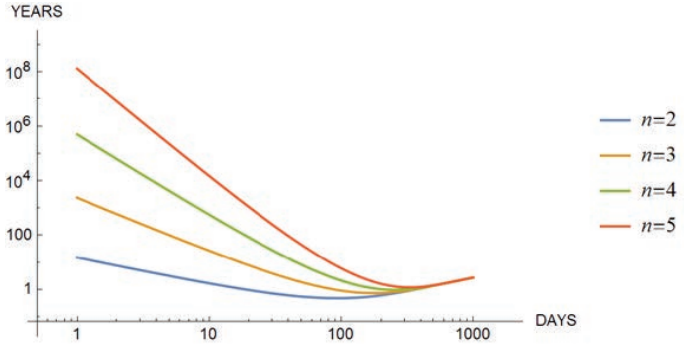
For the case of M22 we have five PCs and multiple NPs. A calculation similar to the above, where it is assumed that volumes of all components add on mixing, is found to give a qv* of 1.34. A more accurate calculation, in which the actual volumetric effects of mixing are taken into account, is shown in the table below; the qv* works out to 1.42. Here we make use of the density of M22, 1.080, so that the amount of water in a liter (1,000 ml; 1,080 g) of solution is the calculations are similar and qv* works out to 1.37, lower than for VM-1, though it appears that both VMs can be considered to have low toxicity in relation to the results shown earlier. For example, rat hippocampal brain slices can be recovered with K/Na ratio's only slightly lower than M22 [10]. More research is needed, both to better assess the severity of damage from existing protocols and to develop better protocols that reduce or eliminate such damage. In any case it appears that the perceptive choice of qv* has accelerated progress in cryoprotectants that might eventually lead to reversible, long-term cryopreservation of tissues and organs, though this goal is still unrealized.

The development of the qv* metric (and the theory of general cryoprotectant toxicity that underpins it) constitutes a major contribution to our understanding of non-specific cryoprotectant toxicity. Further refinements should be possible. For example, as currently calculated, non-penetrating cryoprotectants (like PVP) are considered to be non-toxic, although they do interact with the endothelium and the outer layer of cell membranes. Incorporating the exact hydrogen-bonding strength of the polar groups may further refine qv*. Calculating qv* is only meaningful when cryoprotectant mixtures are chosen with identical vitrification tendencies, but actual toxicity effects can (drastically) change for higher concentrations when a specific threshold is passed to trigger specific toxicity.

Predicting Future Cryonics Case Loads [1,29,30]

Next we consider a very different sort of problem, connected with the fact that cryonics cases are random and unpredictable, so that, for example, relatively long stretches of time can go by between cases, or, on the other hand, there can be several cases over a short time interval. Too many such cases would strain the capabilities of a cryonics organization, so it is desirable to anticipate how often such bunching can be expected.

A main result of some studies by Perry was to develop a formula, based strictly on probability considerations, for the expected waiting time for *n* or more cases to occur over a demand interval of length *t*. Results are shown in the chart below, where curves are plotted for $n = 2, 3, 4$, and 5, assuming a total membership population of 1,000 and an expected average of 7 cases per year, conditions that were fairly approximated at Alcor around 2010-15. (Actually, only the number of cases per year is important, not the member population, but it happens that 7 cases per year is what really occurred for a member population of around 1,000.) We see, for example, that 2 or more cases over a 10-day demand interval *t* could be expected to occur in about a year, whereas (reassuringly) a roughly 30-year wait would be needed for a t of only one day, and 3 or more cases in one day would not be expected for well over 1,000 years. These assumptions cannot be trusted far outside the range of times and other conditions now prevalent and overlook other difficulties, such as pandemics or major accidents which might lead to additional, multiple cases. At any rate, a beginning was made, and some basic results were reassuring.



Expected waiting time (years) as a function of demand interval (days) for n *or more cryonics cases, with* n *ranging from 2 through 5, assuming 7 cases per year for member population of 1,000.*

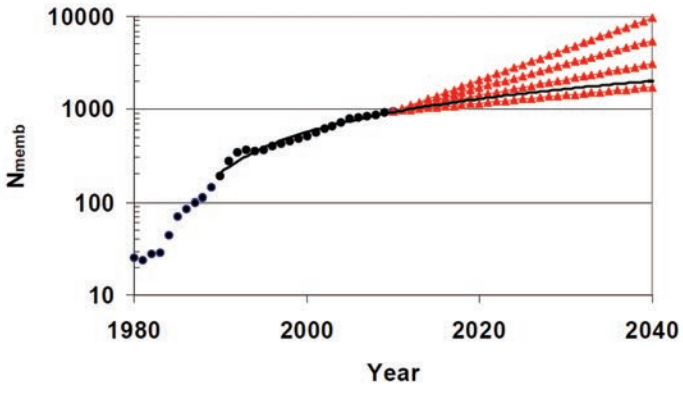
Organizational Stability Issues

One problem not addressed so far, and a very serious one, concerns organizational stability. Some early cryonics organizations were unable to maintain or transfer their patients, which instead were lost (while the organizations themselves subsequently ceased to operate) [28]. Since then much thought and effort has gone into how to prevent the future loss of patients, and particularly, how to ensure that a cryonics organization will be stable and able to maintain its patients indefinitely or until revival is possible. Robert Freitas in a 2010 study addressed this problem for Alcor [35], first noting the financial challenges the organization faced. These included "the current mismatch between cryopreservation funding minimums and actual costs when cryopreservation services are actually rendered, often decades later, combined with Alcor's heavy reliance on bequests and on the continuing generosity of living donors to support core functions."

There had been some "heroic efforts," Freitas noted, to try to address these problems in the past. (Among these was a mathematical study by Perry, noted in passing, estimating how much principal would be needed to fund a cryopreservation indefinitely on interest income, based on different assumptions of rates of return and costs of maintenance [25].) One highly useful tool no one had come up with yet, however, was a "basic top-down econometric model of Alcor's finances." This deficiency Frietas remedies in his study; a footnote explains the field of econometrics and some of its terminology:

> Econometrics is the application of statistical methods to financial or economic data. An econometric model is a set of interlocking mathematical equations that describes the behavior of specific dependent variables when other independent variables fluctuate within their allowed ranges. A "top-down" or "macro" model, as used here, is simplest and can be constructed by starting with top-level aggregated data (e.g., total expenses) and modeling the behavior of that data as a function of several lower-level causative variables (e.g., total number of customers). A "bottom-up" or "micro" model is more complicated (but often more accurate), and may be constructed by starting with low-level unaggregated data (e.g., time series representing actual expenditures on each of 1000 items that the organization must buy in order to provide its services) and then constructing separate sub-models for each of these items, then summing the results of all the sub-models to forecast the top-level aggregated variable(s) of interest. Acquiring the huge amount of data necessary to drive a good bottom-up model can be very expensive and time-consuming.

Due to its complexity, the bottom-up approach that might give the most accurate fit to actual circumstances has been waived in favor of the simpler, top-down approach. Generally, a quantity such as membership, patient total, or expenses will be estimated as a function of time based on linear regression analysis of past data. One example, dealing with Alcor membership, is shown in the figure below; included are projected membership totals up to year 2040 based on various assumptions about growth rates.



Alcor cryopreservation membership (N_{memb}): actual data, 1980-2009 (black dots); regression formula prediction, 1990-2040 (black curve); N_{memb} prediction for 2010-2040 (red triangles), assuming constant +2%/yr (bottom), +4%/yr, +6%/yr, or +8%/yr (top) growth rates.

In a follow-up article in *Cryonics* Freitas summarizes what has been accomplished in his study [36]:

> The analysis starts by creating a model of Alcor's expenses using historical data from 1990-2008. Statistical correlation is employed to predict the expense data using three independent variables: number of members, number of cryopatients, and number of cryopreservations per year. Using various assumed growth rate scenarios for these three independent variables, Alcor expenses can be projected forward 30 years into the future. The analysis continues with the creation of a similar model of Alcor's revenues based on historical data from 1990-2008. Statistical correlation is again employed to predict the revenue data using sub-models for each of Alcor's five principal consolidated revenue sources: (1) dues, (2) standby fees, (3) proceeds from cryopreservations, (4) Patient Care Trust (PCT) earnings, and (5) grants, donations and bequests. Each revenue stream can be predicted using the same three independent variables as before. This allows Alcor's revenues – and, after subtracting predicted expenses, any budget shortfalls or surpluses – to be projected forward 30 years into the future.

Serious concerns are raised about Alcor's then-current fiscal policies. Freitas then offers some recommendations for possible funding options, involving dues increases, cost-of-living adjustments, or donations that would "produce long-term budgetary stability at Alcor." He feels that "members should

always have available to them at least one viable option that includes the permanent grandfathering of their account." Some detailed recommendations for further studies are also presented.

Freitas's work served as an important reference in later discussions and policy decisions on funding issues at Alcor – a lengthy story which can only be hinted at here [2,3].

Cryonics and Machine Learning

Up to now we have considered several applications of basically straightforward numerical mathematics to problems in cryonics. Here instead we will delve into machine learning, a branch of artificial intelligence which considers how a computer algorithm might be modified to improve its performance on tasks of the type that are considered to require intelligence if performed by humans. An example would be a game-playing program where we are interested in the process of generating successively better versions of the program so that the machine "learns" to play better. The learning process could involve human guidance ("supervised learning") or be entirely automated ("unsupervised learning").

Attempts to apply machine learning to cryonics problems include an early study by Perry in which EM images of rat brain cortical tissue, initially represented as 2-D numerical arrays, were collapsed into 1-dimensional "signatures" using Fourier analysis. A primitive machine learning technique was used to find a vector whose inner product with the signature would approximate the amount of ischemic time of the corresponding brain tissue. Results were informally published in 2011 [27]. The intent of the work was to make some headway toward understanding what changes occur in brain tissue as a result of postmortem ischemia. In this way light might be shed on the problem of whether and how cryonics patients might be revived at a future date. Only limited success was achieved, precluding such an ambitious goal, but the new approach opened possibilities for future work.

Recently a second study has been completed [9], that uses a far more sophisticated machine learning technique implemented by Michael Maire to distinguish tissues with different ischemic exposures. The study is important in part for its application of what can be considered state-of-the art deep learning to a problem of cryonics. It is also important for its detailed, systematic study of changes that occur in brain tissue as a function of postmortem ischemic exposure, something that is especially of interest in cryonics but not much pursued outside the field. Perhaps, then, we are closer to the goal of using computational methods to show how important problems connected with cryonics revival could be solved, though much remains to be done before any substantial success can be claimed in achieving such a goal.

For the machine learning application, a deep convolutional neural network is used to addresses a classification task involving electron microscopy (EM) images of rat cortical brain slices (tissue samples). The tissue samples are in two main groups, one exposed to warm ischemia (rat normothermic body temperature, 37°C, similar to human body temperature) the other to cold ischemia (0°C). For the warm ischemia the exposure times range from 0 (control) up to 81 hours. For the cold ischemia the (overlapping, but generally much longer) times are from 0 to 6 months (4,464 hours). (Note that in view of the Q-10 rule we expect a much slower rate of tissue degradation per unit of time at the lower temperature.) A program known as the "computational model" is to be given data derived from an EM image of a tissue sample and return information pertinent to the ischemic history of that sample, that is to say, what temperature the sample was exposed to and for how long.

It turns out that to develop or "train" a computational model that simply outputs this information, and produces reliable answers, would be a very ambitious undertaking at this point. (Indeed, it would require considerable, specialized expertise for a human to carry out such a task, where possible at all. Some of the cases of different ischemic times were hard to distinguish; see below.) So instead a simpler task was chosen for which, initially, the sample images were sorted into batches, with each batch consisting of images of samples which were all alike in times and temperatures. So, one batch might represent controls with no ischemic time at all, another with 1 week (168 hours) of cold exposure (0°C), and so on. Altogether there were n batches of images. (In the results as shown below, $n = 19$, with about 14,000 images per batch. Each image, consisting of a "patch" cropped and downsampled from a larger image, was a 128 x 128 array of pixels showing brain structure, with pixel width about 13 nanometers. Thickness of brain tissue slices as imaged was about 70 nanometers.) To test a given computational model an image is chosen from batch m, say, together with a pair of batch numbers (p, q), in which one of the p, q, is m, the "right answer" and the other is the wrong answer. The computational model is then tasked with making the right choice of batch number among the two possibilities. The performance of the model can be gauged by testing it on a great many examples of images covering all the different combinations of p and q and recording the percentage of correct answers it gives for each combination.

The problem then becomes one of arriving at a computational model that produces correct answers as often as possible. This is the "training" problem. For this we start with a "baseline model" that is totally ignorant of how to answer and gives answers at random, thus achieving 50% correctness (chance only) for each case. During the training phase, when the model gives an incorrect answer a correction is applied to the model to try to improve its performance, and the model in the end should perform well on the training examples. It is then tested on similar examples it was not trained on, images it has not seen before, for each combination of p, q, to see if it will perform well, or at least better than chance, on these cases too. Success

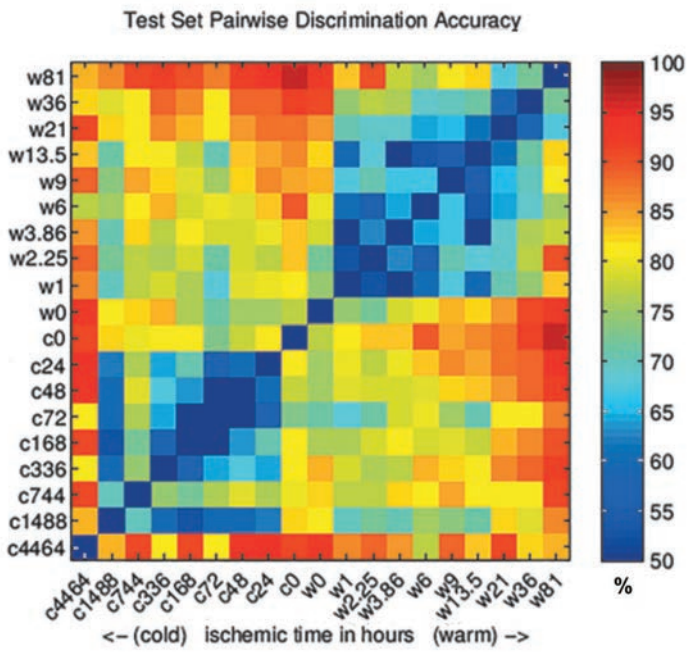
means it has acquired "understanding" of the problem at hand and can impart useful insight.

Additional details of the process are here excerpted; more will be found in the original paper:

> Deep convolutional neural networks have emerged as the standard method for image classification. They are composed of many stacked layers, each consisting of a filter set followed by a nonlinearity and acting on the output of the previous layer. Given an image as input, filters in the first layer typically extract basic structures such as edges, whereas those in deeper layers build more abstract features. Trained on a large dataset of natural images, their learned representations are transferable to a variety of image interpretation tasks. An existing network consisting of 16 convolutional layers trained on natural images was used as a module for generating a descriptor for any generic input image. This network outputs a descriptor in the form of a 4096-dimensional vector produced by its deepest layer.
>
> Within this computational framework, we explored the question of the discriminability of electron microscopy images of samples subjected to different ischemic time and temperature conditions. … Tested on an equal number of images from each of … two conditions [that is, (*p*,*q*) where first *p* then *q* is the right answer], the baseline model (random chance) would have an accuracy of 50%. The extent to which a trained model is capable of outperforming chance is indicative of the visual distinctiveness of neural tissue subject to the two different experimental conditions. All possible pairs of ischemic conditions were considered, and a discriminative model was trained for each pair.

Principal results are shown in the chart below, for nineteen batches of images covering both the cold and warm ischemia cases as noted above, each batch label beginning either "c" ("cold") or "w" ("warm") followed by number of hours. We start with the outlier case of cold ischemia (c4644, batch 1), count downward through the time values to c0 (batch 9), then upward through the warm time values to w81 (batch 19). For each (*p*,*q*) pair of possible batches we show the color-coded percentage of correct answers of the trained computational model when first *p* then *q* is the right answer. Thus the overall result for (*p*,*q*) will equal that for (*q*,*p*); in the chart the results are shown only for the values above chance with neutral fill-in (50%, chance level) otherwise, and in the trivial case of *p*=*q*.

Among the interesting details is that, as expected, ischemic times that are close together with similar temperatures are generally not as strongly distinguished as cases with greater disparity, some nearby cases approaching or (in the chosen representation) equaling chance only. Another interesting property is that most of



the cold ischemia cases are rather strongly distinguished from the warm cases (upper-left and lower-right quadrants), regardless of exposure times, and better distinguished than cases within each group are from each other (lower-left and upper-right quadrants). It appears that rather different types of distinguishable damage occur at the two different temperatures. A third observation is that controls tend to be especially well-distinguished from all non-controls, suggesting that some important damage occurs early on; for example, in the case of warm ischemia, with E-HIT of 1 in the first hour. The ability of the algorithm to successfully distinguish between the very early stages of ischemia and the late (necrotic) stages of ischemia appears to support the practice of cryonics, which takes advantage of the fact that there is an intermediate "island of ultrastructural stability" (lasting for hours at normal body temperature) which permits preservation of identity-critical information. Overall, then, the work may be an opening wedge that could lead to greater application of machine learning and/or more general artificial intelligence in cryonics problems, with exciting conclusions.

Closing Remarks

In cryonics we are concerned with whether our procedures will be good enough for the hoped-for revival of our patients someday. We must do the best we can without knowing the answer to this all-important question. The driving interest we have in ensuring our procedures are "good enough" without knowing at what point we may have succeeded impels us to examine and analyze our procedures closely, and it might be argued that this is mainly where mathematics comes into play.

We have seen how mathematics was used decades ago to help understand and address problems of cryoprotective perfusion

and heat flow. Some years later the problem of ischemic injury prior to cryogenic cooling came under scrutiny, with a metric, the E-HIT, proposed to estimate the amount of injury resulting from different possible cryopreservation protocols. Meanwhile some studies were done that modelled cryoprotectant toxicity to arrive at better vitrification solutions for cryogenic cooling. A study was done to estimate the likelihood of excessive cryonics caseloads based on an assumption that cases occur at random. Alcor finances came under scrutiny in another study, with long-term projections of expenses and revenues under different assumptions of funding requirements and likely caseloads. Finally, there is the recent study in which an attempt is made to apply machine learning to gain a computerized understanding of the progress of ischemic damage before sufficient cooling can take place.

Overall, there is much that remains to be done. There is also much that *has* been done of a more routine nature, connected with the automation of perfusion and cooldown procedures, for example, that was not reported here (notwithstanding the considerable and diligent efforts that were involved). Many of the mathematical studies reported here do not appear to have had any profound effect on policies and protocols to date. Some notable exceptions were the studies on cryoprotectants and the studies about the long-term effects of "grandfathering." This situation will hopefully improve in the future when we may expect that deeper mathematical studies can be conducted, again possibly involving artificial intelligence. Meanwhile we can look for relatively simple ways to improve our protocols; further development of E-HIT metric and combining them with CT scan results might be one good place to start. ■

The authors thank Greg Fahy and Brian Wowk for assistance, particularly with the section on cryoprotectants Hugh Hixon also provided some helpful comments on current cryonics procedures regarding early-stage cooling.

Image Credits

Images relating to heat flow and perfusion modeling were taken from the references cited and sources should be clear. Plots of "% of control K^+/Na^+ vs. qv*" were based on graphics from [17]. The graphic showing Alcor membership and projected membership over time was adapted with permission from the reference cited ([35]); "Test Set Pairwise Discrimination Accuracy" was based on graphics from [9]; artwork by RMP unless otherwise noted.

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2019 Aging Conferences

By Ben Best

Background

Major recent advances in aging research have been so dramatic that many age-reversal therapies are becoming increasingly accessible. Some people are even self-experimenting with therapies before safety has been established in clinical trials. Three noteworthy examples will be described in detail.

With aging, cells increasingly become malfunctional due to increasing DNA damage, especially due to shortening chromosomes (telomere shortening). These aged cells (senescent cells) are not only malfunctional, but have damaging effects on other cells. Removal of senescent cells can have rejuvenating effects, and one drug that does this is the prescription anti-cancer drug dastinib. Dastinib can reduce immune system cells, cause rash, and could harm the baby of a pregnant woman. Nonetheless, in most cases the drug is safe, especially when used only occasionally to eliminate senescent cells, rather than when used chronically to eliminate cancer cells.

Nicotinamide adenine dinucleotide (NAD+) is an essential coenzyme required for the activity of sirtuins[1] and the PARP (poly-(ADP-ribose) polymerase) enzymes used for DNA repair.[2] When enzymes use NAD+, the NAD+ is converted to nicotinamide (NAM), which is either recycled to produce NAD+ or is methylated for excretion in the urine.

There are seven human sirtuins (SIRT1-SIRT7) requiring NAD+ for stem cell function (maintaining healthy tissues),[3] for DNA Repair,[4] and for function of the energy-producing mitochondria in cells. Sirtuins for good mitochondrial function are especially important in organs requiring large amounts of energy, including the heart[1] and the brain.[5] Sirtuins activated by NAD+ inhibit the chronic inflammation associated with many aging-related diseases.[6]

Because NAD+ declines sharply with age,[7] some people have taken intravenous NAD+ infusions, a process often associated with intense abdominal pain. Stress on the kidney or liver (possibly causing damage) might be the cause of this pain, but there has been no scientific investigation of this problem. Methylation is required to excrete the NAD+ waste product nicotinamide in the urine, a process which depletes the methylating agent s-adenosyl methionine, making it unavailable for other methylations.[8] Although NAD+ at higher levels is beneficial to cells in general, it is also beneficial to senescent cells, worsening their damaging effects.[9]

Because NAD+ is a charged molecule, some scientists have thought that NAD+ cannot cross cell membranes. But one study found that specialized ion channels in neurons allow NAD+ to enter these brain cells,[10] and another study found that these ion channels are not restricted to neurons.[11] A study using radiolabeled NAD+ found that NAD+ was transported into six cell lines (fibroblast, neuroblastoma, HeLa, keratinocytes, macrophages, and endothelial cells).[12] A pilot study of eleven human males (8 receiving NAD+ infusions, 3 controls) indicated tissue absorption of NAD+ during the first two hours, but after the first two hours the NAD+ (or nicotinamide) was mostly being excreted in the urine.[13]

Metformin has been shown to reduce cellular senescence in cells subject to senescent-causing stresses.[14,15] Metformin also protects cells against inflammation due to Advanced Glycation End-products (AGEs).[16] Metformin has been shown to promote brain cell genesis and improve memory in mice.[17] Metformin also has health benefits by improving microbiota in the gut.[18,19] In a very large clinical trial, type 2 diabetics treated with metformin lived 15% longer than non-diabetic subjects not taking metformin.[20]

One reason why type 2 diabetics have elevated blood glucose is that their livers produce glucose at twice the rate seen in normal subjects, but metformin reduces this glucose production by one-third.[21] Metformin interferes with the distribution of iron in cells, a problem that can be partly corrected by taking iron supplements.[22]

The main sensor of cellular energy is AMPK (AMP-activated Protein Kinase).[23] When cellular energy is depleted (low ATP, high AMP from ATP), AMPK reduces energy-utilizing metabolism and increases processes that produce the high energy ATP molecule.[23] Recycling of damaged proteins for energy (autophagy) is one of the AMPK processes that is stimulated.[23] AMPK increases NAD+ and sirtuin activity, which increases DNA repair and reduces chronic inflammation.[24] Although metformin has been shown to activate AMPK in a variety of experiments, the amount of metformin required is much less than a person taking metformin would achieve in the usual doses.[25,26] AMPK can be activated by lipoic acid,[27] vinegar,[28] resveratrol,[29] curcumin,[29] as well as by exercise and calorie restriction.[29]

A large clinical trial of nondiabetic persons comparing metformin with lifestyle (moderate exercise and diet) showed that lifestyle reduced the incidence of type 2 diabetes 58% over a 2.8-year period whereas metformin reduced the incidence by 31%.[30]

People exercising and taking metformin showed a lessened increase in insulin sensitivity and endurance capacity[31,32] and a reduced increase in lean muscle mass.[33]

This report focuses on presentations relevant to age-reversal made at 2019 aging-related conferences.

CD38 Degradation of NAD+

Eduardo Chini, MD, PhD (Assistant Professor of Medicine, Mayo Clinic College of Medicine, Rochester, Minnesota) identified CD38 enzyme as the main enzyme degrading NAD+ with aging.[34] Mice in which CD38 enzyme has been removed have 10 to 20 times more NAD+ than normal mice.[35] CD38 not only degrades NAD+, but also degrades the NAD+ precursors NR (nicotinamide riboside) and NMN (nicotinamide mononucleotide).[36] Prior to Dr. Chini's discovery, it was believed that NAD+ declines with age mainly due to NAD+ consumption by PARP to repair the increasing age-associated DNA damage.[37]

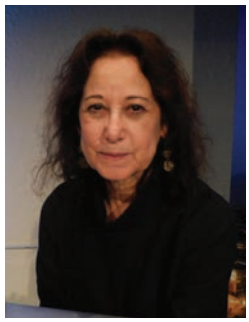
Aging and obesity results in increased visceral fat, which becomes infested with CD38-laden macrophages (immune system cells) that massively consume NAD+ while also causing chronic inflammation (https://www.biorxiv.org/content/10.1101/609438v1.full.pdf). Secretions from senescent cells also increase the amount of CD38 found on macrophages.[38] Dr. Chini has proposed using CD38 inhibition to treat symptoms of the metabolic syndrome (pre-diabetes).[39] Dr. Chini has also shown that the increasing CD38 (and declining NAD+) with age is associated with declining mitochondrial function.[34]

TIME+ to Boost NAD+

Nichola Conlon, PhD (CEO, Nuchido Limited, Newcastle, United Kingdom) has co-founded a company (Nuchido) to produce a supplement (TIME+) which increases NAD+ by a combination of mechanisms. Normally, the main source of NAD+ in cells comes from the enzyme that recycles the breakdown product of NAD+ (NAM, nicotinamide) resulting from NAD+ use by sirtuins, PARP, and CD38.[40]

TIME+ includes rutin, a quercetin derivative that both stimulates the NAD+ recycling enzyme41 and inhibits CD38.[39] TIME+ also includes apigenin to inhibit CD38,[39] and green tea extract (EGCG, epigallocatechin gallate) to inhibit the enzyme that excretes NAM in the urine. Supplementation with the NAD+ precursors NR and NMN are other means of boosting NAD+,[42] but Nuchido has evidence that TIME+ boosts NAD+ about four times more than a precursor. Nuchido is conducting clinical trials with TAME+. TIME+ also contains NAM on the assumption that NAM will quickly increase NAD+ because the recycling enzyme is activated. But NAM will potently inhibit sirtuins if NAM is not quickly recycled.[43]

Damaging Products of Senescent Cells



Judith Campisi, PhD (Professor, The Buck Institute for Research on Aging, Novato, California) is a leading authority on senescent cells. Senescent cells are cells that can no longer grow, are resistant to self-destruction (apoptosis), and which have toxic secretions.[44] Dr. Campisi coined the term SASP (senescence-associated secretory phenotype) to describe the characteristic of senescent cells that cause secretion of inflammatory proteins, growth factors, and protein-degrading enzymes.[45] Senescent cells remain metabolically active,[46] and can even cannibalize neighboring cells.[47] Having low levels of autophagy, senescent cells can accumulate lipofuscin (cellular garbage often seen in brain cells),[48] which can be seen in age spots on the skin of older persons.[49]

Dr. Campisi has helped to show that senescent cells accumulate in visceral fat, contributing to the activation of the CD38 enzyme which degrades NAD+ (https://www.biorxiv.org/content/10.1101/609438v1.full.pdf)

When cells become senescent they stop dividing, which can prevent the cells from becoming cancerous.[50] Cellular senescence prevents cancer early in life, but later in life increasing amounts of SASP factors promote cancer.[51] Chronic inflammation due to SASP contributes to atherosclerosis, diabetes, and cancer.[45] Although aging, DNA damage, and obesity are the main causes of cells becoming senescent, environmental toxins can also induce senescence.[52]

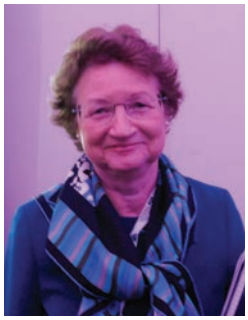
Therapy to Remove Senescent Cells

James Kirkland, MD, PhD (Professor of Medicine, Mayo Clinic, Rochester, Minnesota) is the foremost researcher applying senolytic therapy (therapy to eliminate senescent cells) in clinical trials. Senolytic therapy is difficult because of the wide variety of senescent cell types,[53-55] which means that no single senolytic agent can target all senescent cells.[56] Dr. Kirkland has conducted a number of pilot studies (small number of patients) using dastinib plus quercetin to reduce senescent cells from diabetic patients,[57] from patients with idiopathic pulmonary

fibrosis (improving physical function),[58] and from pregnant women suffering from preeclampsia (high blood pressure with vascular disease).[59] That last study was even shown to remove senescent cells (improving stem cell function) in the pregnant control subjects who did not have preeclampsia.[59]

In a testing of ten flavonoids (including fisetin, rutin, curcumin, and quercetin), fisetin was found to be the most potent senolytic.[60] Dr. Kirkland is conducting a clinical trial (NCT03430037, ClinicalTrials.gov) to determine if fisetin can reduce frailty and inflammation in elderly women.

First FDA-Approved Therapy for a Genetic Diseases



Katherine High, MD (President and Chief Scientific Officer, Spark Therapeutics, Philadelphia, Pennsylvania) co-founded a company that developed the first gene therapy for a genetic disease approved by the US Food and Drug Administration (FDA).[61] A mutation of the RPE65 gene results in visual impairment from infancy, and eventual blindness in adulthood.[61] For delivery of the gene to correct this condition, Dr. High's company selected an adeno-associated virus (AAV), a virus which is regarded as relatively safe because the AAV DNA does not become inserted into the patient's DNA (which could risk causing cancer).[62]

Nonetheless, up to 70% of people have antibodies to AAV (prevalence increases with age), and these antibodies can interfere with gene delivery.[63] Two factors reduce the risk of immune system rejection of RPE65 gene therapy with AAV: (1) the gene therapy is administered directly to the retina, a tissue which does not readily develop an immune response, and (2) the immune suppression drug prednisone is used in conjunction with the gene therapy (over a period of a few weeks).[64]

In December 2017 the FDA approved Luxturna, the gene therapy created by Dr. High's company Spark Therapeutics.[65] Most of the patients have shown significant benefit (especially the youngest ones), and most of the adverse effects have been secondary to the surgical procedure required to administer Luxturna directly to the eye.[65]

Gene Editing and Gene Therapy for Health and Longevity

George Church, PhD (Professor of Genetics, Harvard Medical School, Boston, Massachusetts) is attempting to use gene editing and gene therapy to extend health-span by making organisms resistant to all viruses, by modifying pig genes so pig organs can be transplanted into humans,[66] and by reversing aging using genetic engineering.[67] His company Editas is using AAV to deliver CRISPR-Cas9 gene editing technology to the retina to correct mutations in the CEP290 gene that causes blindness.[68]

Dr. Church's teams are doing research to improve targeting of AAV to the liver[69] and to use microRNA molecules to reduce immune reactions to AAV.[70] The Cas9 enzyme of CRISPR-Cas9 gene editing causes an immune response, so means of preventing immune rejection of CRISPR-Cas9 is also Dr. Church's goal.[71]

Dr. Church has used AAV gene therapy to deliver three longevity associated genes to the livers of mice (FGF21, α-Klotho, and TGFβ1).[72] The mice showed a 58% increase in heart function, a 75% reduction in kidney atrophy, and complete reversal of obesity and insulin resistance associated with a high-fat diet.[72] Unexpectedly, the AAV: α-Klotho interfered with the effectiveness of the AAV:FGF21.[72] Dr. Church's company Rejuvenate Bio (rejuvenatebio.com) is applying a version of the therapy excluding the α-Klotho to testing with dogs (with the hope of later use on humans if safety and effectiveness are proven on the dogs).

Retrotransposons Cause DNA Damage and Inflammation

John Sedivy, PhD (Professor of Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, Rhode Island) studies the effect of retrotransposons ("jumping genes") on health and longevity. Retrotransposons compose up to two-thirds of the human genome.[73] Retrotransposons are DNA segments that can self-replicate, and insert themselves at different places in the genome causing DNA damage and sometimes cancer.[74] Retrotransposons are parasites of the genome that are an important cause of cellular senescence.[75]

Retrotransposons are mostly repressed early in life, but become increasingly active with aging, leading to increasing DNA damage, inflammation, and cellular senescence.[76] In young animals retrotransposons are repressed by specialized RNA molecules,[76] by the cancer suppressor protein p53,[77] by the sirtuin protein SIRT6,[78] and by the anti-virus protein interferon.[79] Senescent cells can be removed by a healthy immune system, a process which depends upon interferon.[80]

Dr. Sedivy led a study which found that retrotransposons can escape the cell nucleus, causing interferon to respond to the retrotransposons as if they were virus, resulting in chronic inflammation.[73] This happens especially in senescent cells, causing the senescent cells to become an even greater source of inflammation than they would be due to SASP.[73]

Autophagy Prevents Disease

David Rubinsztein, PhD (Professor, University of Cambridge, Cambridge, United Kingdom) studies the effect of autophagy on health and longevity. Autophagy is a means by which cells digest damaged cellular components, which are recycled to provide energy and new cell components.[81] Autophagy declines with age, partly because the autophagy-suppressing protein mTOR increases with age.[82]

Autophagy is particularly critical in brain cells, because when protein aggregates accumulate rather than being recycled, the aggregates can contribute to Alzheimer's Disease, Huntington's Disease, or Parkingson's Disease (among others).[83,84] Defective mitochondria can also lead to those diseases. Digestion of defective mitochondria by autophagy declines with age due to the decline in NAD+ and sirtuin activity.[85,86] Impaired autophagy leads to senescence of immune system cells.[87]

Administration of the autophagy-stimulating polyamine spermadine to mice enhances immune system function.[88] Administering the mTOR inhibitors rapamycin or spermidine (which, unlike rapamycin, does not suppress the immune system) reversed senescence of stem cells by enhanced autophagy, thereby restoring the capacity of tissues to regenerate.[89,90] Exercise and fasting also increases autophagy.[91]

Sirtuins to Improve Stem Cell Function

Danica Chen, PhD (Associate Professor of Metabolic Biology, Nutritional Sciences & Toxicology at University of California at Berkeley) investigates the stem cell theory of aging, which holds that stem cell failure with increasing age results in the incapacity to maintain tissue function.[92] She has a particular interest in hematopoietic stem cells (HSCs), the stem cells that give rise to all blood cells, including immune system cells and red blood cells. She suggests that sirtuins have the capacity to reverse stem cell aging.[92] Increasing the sirtuins SIRT3 or SIRT7 rejuvenates aged HSCs.[93]

Dr. Chen has shown that SIRT3 maintains mitochondrial function in HSCs[94] and that SIRT7 reduces stress from misfolded proteins in mitochondria.[95] She has shown that SIRT2 opposes HSC aging by suppressing inflammation in the HSCs.[96] Dr. Chen acknowledges that sirtuin activity is dependent upon NAD+ (which declines with age). She suggests that declining sirtuin activity results in declining gene regulation, causing defective HSCs.[97]

A Blood-Based Biomarker of Aging

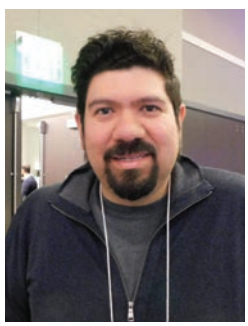
Morgan Levine, PhD (Assistant Professor of Pathology, Yale School of Medicine, New Haven, Connecticut) did postdoctoral work with Dr. Steve Horvath, identifying DNA methylation sites that are associated with chronological and biological age.

All the cells of the body contain the same DNA, but different tissues express different parts of the genome. Different tissues express different genes, partly because gene expression can be prevented either by compaction of unneeded parts of the genome, or by the addition of methyl groups to DNA. Methylation of DNA generally declines with age (including reduced repression of retrotransposons), although methylation often increases in DNA regions controlling gene expression (promoters).[98]

Dr. Horvath pioneered measures of DNA methylation as a biomarker of aging.[99] Dr. Levine developed a biomarker of aging (DNAm PhenoAge) based on both methylation status and blood-based measures of health (including glucose levels, inflammatory proteins, white blood cell count, etc.).[100] Dr. Horvath's methylation biomarker is based on 30 different tissue and cell types, whereas Dr. Levine's biomarker only uses blood cells and plasma components (simplifying measurement).[101] But Dr. Levine's blood-based method has been shown to strongly correlate with chronological age in many tissues and cells.[100] Dr. Levine's methylation clock is superior to Dr. Horvath's in predicting time to death as well as smoking status and immune system aging.[101]

Some researchers have suggested that rejuvenation could be accomplished by DNA methylation modification.[98] The lifespan of fruit flies has been extended by this method.[102]

Heterochronic Parabiosis to find Aging Factors in Blood



Saul Villeda, PhD (Assistant Professor, Department of Anatomy, University of California, San Francisco) studies the effect of sharing blood circulation between genetically identical mice of different ages (heterochronic parabiosis) on brain function. He has shown that heterochronic parabiosis improves learning and memory in old mice.[103] Sharing of circulation may dilute inflammatory factors of old mice. Heterochronic parabiosis between 2-month old mice and 15-month mice had no negative effects on cognitive function of the young mice, but a dramatic inhibition of brain function in

young mice was seen when 21-month-old mice were used for parabiosis.[104]

Dr. Villeda has sought to identify blood factors causing positive effects in old mice, and negative effects in young mice—and has found some of those factors related to inflammation.[105] Daily injection of the serum protein GDF11 into old (21-23 month) mice for 4 weeks restored brain cell genesis in the old mice.[104] Subsequent experiments established that GDF11 cannot enter the brain from the blood circulation, suggesting that the benefit from GDF11 is on the blood vessels of the brain.[106]

An FDA-Approved Clinical Trial for Anti-Aging Therapy

Nir Barzilai, MD (Professor, Albert Einstein College of Medicine, Bronx, New York) is the lead investigator of a clinical trial to reduce aging with the prescription anti-diabetic drug metformin (the TAME trial, Targeting Aging with MEtformin). TAME is designed to enroll 3,000 subjects aged 65 to 79 to take metformin or a placebo for 6 years.[107] The TAME trial was approved by the FDA in 2015, but there were not sufficient funds to conduct the trial until an unidentified donor added enough money in 2019 to make the trial financially feasible. TAME is unique for an FDA-approved clinical trial in that metformin is not being evaluated to treat a single disease, but is being used to prevent a composite of cancer, dementia, mortality, and cardiovascular disease, as well as general function of the subjects.[108] Although one of the objectives of the trial is to create a precedent for FDA-approved trials that target aging rather than a specific disease, critics suggest that preventing a new aging-related disease does not qualify as slowing aging.[109] In fact, metformin failed to extend lifespan in a multi-centered test on mice, unless it was combined with rapamycin.[110]

Concluding Remarks

In contrast to the situation even a decade ago, recent aging research is discovering means of reversing aging. Cautious scientists await extensive clinical trials before wanting these therapies to be applied to humans, whereas other people are eager to try these therapies immediately, even at the risk causing harm. ■

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Fight Aging!

Reports From the Front Line in the Fight Against Aging

Reported by Reason

Fight Aging! exists to help ensure that initiatives with a good shot at greatly extending healthy human longevity become well known, supported, and accepted throughout the world. To this end, Fight Aging! publishes material intended to publicize, educate, and raise awareness of progress in longevity science, as well as the potential offered by future research. These are activities that form a vital step on the road towards far healthier, far longer lives for all.

Building a Biomarker of Aging from Frailty Measures

January, 2020

A biomarker of aging is a way to measure biological age, the burden of cell and tissue damage and consequent dysfunction. A biomarker that permitted the robust, quick, and cheap assessment of biological age would greatly speed up development of rejuvenation therapies. It would allow for rapid and cost-effective tests of many interventions, and the best interventions would quickly rise to prominence. At present the rigorous assessment of ways to intervene in the aging process is slow and expensive, as there is little alternative but to run life span studies. Even in mice that is prohibitively costly in time and funds for most research and development programs.

One of the more severe consequences of this state of affairs is that it takes a long time and sizable expense to weed out the less effective approaches to treatment. That this is a problem is well recognized by the scientific community, and many varied biomarkers of aging are presently under development. Perhaps the best known are the various forms of epigenetic clock, weighted algorithmic combinations of the status of DNA methylation sites that correlate with age and mortality risk. There are other approaches, though, such as combining simple measures of decline such as grip strength or inflammatory markers in blood tests. That class of methodology is explored in today's open access paper, with the focus specifically on measures adopted by the clinical community to assess frailty.

One of the concerns with the epigenetic clock, and for similar efforts using levels of blood proteins, is that it is quite unclear as to what exactly is being measured. The relationship with age and mortality emerges from the data, and it is then up to the research community to establish mechanistic connections between specific epigenetic changes and underlying processes of aging. It is quite possible that these biomarkers do not reflect all of the mechanisms of aging, and thus any use of them to assess a specific approach to rejuvenation would have to be carefully validated in parallel with the development of that therapy. This somewhat defeats the point of the exercise. When building a biomarker based on frailty indices, as here, there is at least a greater degree of confidence that it comprehensively touches on all of the contributions to aging, and we would thus expect any viable rejuvenation therapy to make a difference to the measure of age.

Biological age is an increasingly utilized concept that aims to more accurately reflect aging in an individual than the conventional chronological age. Biological measures that accurately predict health and longevity would greatly expedite studies aimed at identifying novel genetic and pharmacological disease and aging interventions. Any useful biometric or biomarker for biological age should track with chronological age and should serve as a better predictor of remaining longevity and other age-associated outcomes than does chronological age alone, even at an age when most of a population is still alive. In addition, its measurement should be non-invasive to allow for repeated measurements without altering the health or lifespan of the animal measured.

In humans, biometrics and biomarkers that meet at least some of these requirements include physiological measurements such as grip strength or gait, measures of the immune system, telomere length, advanced glycosylation end-products, levels of cellular senescence, and DNA methylation clocks. DNA methylation clocks have been adapted for mice but unfortunately these clocks are currently expensive, time consuming, and require the extraction of blood or tissue.

Frailty index assessments in humans are strong predictors of mortality and morbidity, outperforming other measures of biological age including DNA methylation clocks. Frailty indices quantify the accumulation of up to 70 health-related deficits, including laboratory test results, symptoms, diseases,

and standard measures such as activities of daily living. The number of deficits an individual shows is divided by the number of items measured to give a number between 0 and 1, in which a higher number indicates a greater degree of frailty. The frailty index has been recently reverse-translated into an assessment tool for mice which includes 31 non-invasive items across a range of systems. The mouse frailty index is strongly associated with chronological age, correlated with mortality and other age-related outcomes, and is sensitive to lifespan-altering interventions. However, the power of the mouse frailty index to model biological age or predict life expectancy for an individual animal has not yet been explored.

In this study, we tracked frailty longitudinally in a cohort of aging male mice from 21 months of age until their natural deaths and employed machine learning algorithms to build two clocks: FRIGHT (Frailty Inferred Geriatric Health Timeline) age, designed to model chronological age, and the AFRAID (Analysis of Frailty and Death) clock, which was modelled to predict life expectancy. FRIGHT age reflects apparent chronological age better than the frailty index alone, while the AFRAID clock predicts life expectancy at multiple ages. These clocks were then tested for their predicitve power on cohorts of mice treated with interventions known to extend healthspan or lifespan, enalapril and methionine restriction. They accurately predicted increased healthspan and lifespan, demonstrating that an assessment of non-invasive biometrics in interventional studies can greatly accelerate the pace of discovery.

Link: https://www.biorxiv.org/content/10.1101/2019.12.20.884452v1

The Concept of Successful Aging is Harmful to Research and Development

January, 2020

As illustrated in this research commentary, all too many researchers continue to view aging as something distinct from age-related disease, and this inevitably leads to a poor approach to research and development. In this case, it is a rejection of the idea that rejuvenation is possible in principle at the present time. If one believes that aging and age-related disease are distinct, then one can also think that it is possible to age successfully, or age healthily. That we should split out the concepts of aging and disease, and only treat disease. This is all abject nonsense. There is no such thing as healthy aging or successful aging. There are processes of aging that can clearly be reversed, either actually or in principle. Too many people in positions of influence are producing irrational strategies for medical research under the belief that healthy aging is a viable goal.

Aging is by definition the accumulation of damage and dysfunction that raises mortality risk over time; it is a process of harm and loss. A "healthy" 80-year-old is in no way healthy by any objective measure. Can he sprint the way he used to? No. Is his hearing and eyesight the match of a youngster? No. Are his arteries damaged and distorted? Yes. Does he have a mortality risk that would raise eyebrows in a 20-year-old? Also yes. This is not health. This is a considerable progression towards the polar opposite of health.

To call any particular outcome of the damage and dysfunction at the roots of aging a disease is to draw an arbitrary line in the sand and say that some dysfunction is healthy, and won't be treated, while a little more dysfunction than that is unhealthy, and a disease that should be treated. Sadly this is exactly how medical science has progressed for all too long, even as the scientific understanding of aging needed for a better approach was assembled over the past century or more. The outcomes on either side of that arbitrary line in the sand (yes, you have clinical arthritis and will be treated, versus no, you have signs of progression towards clinical arthritis and come back later) all result from the same processes of damage taking place under the hood. This damage grows with time and leads inexorably to organ failure and death. Thus we should develop rejuvenation therapies to repair that damage, ideally long before it rises to the level of causing pathology. History teaches us that any other path is doomed to failure at worst and marginal, accidental gains at best.

Are We Ill Because We Age?

In the optic of geroscience, if aging becomes a treatable disease/process, it will be the duty of medical doctors to treat it. However, not everything which seems to be aging is aging. Over the history of gerontology and geriatrics, many processes previously thought to be part of aging are now considered not to be age-related, but an overlaying pathology. One of the best examples is anemia, which for decades was considered as a solid attribute of aging but now is considered related to various pathologies and not to aging itself. So, an older individual who does not have relevant underlying pathomechanisms would not have anemia even at 100 years of age or more. The same applies to hypertension, to sarcopenia, to kidney failure, and to cognitive impairment.

So again, what distinguishes aging from a disease conceptually? First, the extent of aging is systemic and complex while that of a disease is mostly limited. Aging is an inevitable, universal process (concerning all humans living long enough) while most diseases are associated with individuals' susceptibilities/vulnerabilities, and most of them, even chronic, are preventable. The most important cause of aging is time, while diseases usually have specific known causes. In other words, aging is irreversible and progressive while diseases are reversible and discontinuous. Finally, and most importantly, aging may be modulable but not treatable, while diseases are ultimately treatable even if we do

not know presently how, which is only a question of progress of science. So many essential differences clearly speak against the notion that aging is "just another" disease.

We should ask how we would know if an anti-aging therapy really could slow aging. The problem is that most of our definitions are circular or impractical. At the most macro level, we might ask whether it extends lifespan or life expectancy. We might ask if we reduce the incidence or burden of age-related diseases (ARDs) with anti-aging interventions. However, it is possible we could do this by counteracting negative aspects of modern lifestyle (e.g., obesity), without affecting aging per se, and conversely that we might find interventions that slow aspects of aging without having much impact on ARDs. Lastly, we might ask whether anti-aging interventions have impacts on metrics of biological aging. If these metrics are specific metrics of the processes being treated, the reasoning becomes circular. For example, we could not prove that senolytics affect aging simply because they reduce the number of senescent cells. Higher level indicators of biological age, such as homeostatic dysregulation indices or the epigenetic clock, are slightly more promising metrics. However, even here there is a problem: these various indices are only poorly correlated with each other and are themselves based on various theories about what aging is. For example, if senolytics lower (rewind) the epigenetic clock, is this simply because the epigenetic profiles of senescent cells are different, and we have removed these cells from the mix? Or was there really an impact on aging in the remaining cells?

At this stage of our knowledge there is no place in medicine for anti-aging medicine understood as treating symptoms of aging when aging has already happened. However, there might be a place for interventions/modulations that would delay the occurrence of aging, when applied early in life, before any time-dependent processes had accumulated and aging symptoms show up. Scientists should recognize at this stage that we know a lot but not enough yet to translate the scientific discoveries in the field of gerontology to interventions into the older subjects. However, a new approach is needed and should be oriented at a systemic conceptualization of the aging process and not at the fragmentation of its different components.

Thus, better assessment of biological aging versus chronological aging holds promises to be able (e.g., by significant biomarkers) to assess the physiological aging processes in their complexity and act on them specifically and jointly. The concept that aging does not always lead to ARD, but that the same processes may lead to either ARD or successful aging in older persons depending on the homeodynamics, will also help to individualize the interventions. Furthermore, the recognition that not everything occurring in aging is detrimental will help to design purposeful interventions to reinforce what is necessary and combat what IS detrimental. Finally, the recognition of aging as a lifelong process and that chronic diseases start early in life would help to design interventions very early in life having consequences on ARD. So, we should move from the notion of aging as a disease concept to aging as an adaptation, which may result in ARD or successful functional healthspan.

Link: https://www.frontiersin.org/articles/10.3389/fphys.2019.01508/full

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Notes on the 2020 Longevity Therapeutics Conference in San Francisco

February, 2020

I recently attended the 2020 Longevity Therapeutics conference in San Francisco. I presented on the work ongoing at Repair Biotechnologies, but as is usually the case the more important parts of the visit took place outside the bounds of the conference proper. Longevity Therapeutics is one of the four or five core conferences for the longevity industry, at which you'll meet many of the early participants – a mix of scientists, entrepreneurs, investors, and patient advocates. As such, most of the conference goers have already seen my updates, or are otherwise aware of the Repair Biotechnologies programs aimed at thymic regeneration and reversal of atherosclerosis. This year was heavily biased towards the entrepreneurial component of the community. It was even the case that most of the scientists attending were presenting in the context of a company that is advancing their work towards the clinic. As the longevity industry expands, ever more researchers in the aging field are finding the opportunity to start a company, or otherwise hand off their work for clinical development.

The first day was a lightly populated set of workshops prior to the conference proper. In the morning, Aubrey de Grey of the SENS Research Foundation and AgeX Therapeutics gave his usual overview of the state of rejuvenation research and development, with a little more emphasis than usual on clinical development and investment in the field. Irina Conboy discussed the plasticity of aging; she is one of the more noted researchers involved in the modern investigations of parabiosis, in which old and young mice have their circulatory systems linked. She gave a tour of differences observed in old mice during parabiosis, such as improved liver regeneration. The argument of beneficial factors in young blood versus detrimental factors in old blood has resolved, by the sound of it, to the conclusion that both mechanisms are relevant – there are a lot of different factors, of different importance. She noted that she is starting a company to push forward some of her work on upregulation or downregulation of factors identified in parabiosis, particularly the combination of oxytocin and TGF-β. Michael Fossel talked about the hallmarks of aging and what to do with them. His point was that metabolism and aging are enormously complicated, forming a system that exhibits risk factors rather than deterministic behaviors. The focus should be on finding the best

point of intervention, which is not the same thing as understanding the system. Greater understanding only makes finding the best point of intervention easier, it isn't absolutely required.

The afternoon was more focused on clinical translation, with presentations from companies further along in the process of conducting trials with the FDA. Mark Allen of Elevian talked about indication choice as a challenging process for companies targeting aging. Elevian is a GDF11 company, and they presently think that prior issues with contradictory results for GDF11 delivery in animal models were due to poor manufacture of the protein. Indication choice is challenging for therapies intervening in aging because so many different indications can be considered, but most are dead ends. It is very important to consider how the choice of indication affects time to market: one is looking for short treatments that can produce large effects. Further, if you want the FDA on your side, you really have to go after large unmet needs for serious diseases. The Elevian team used a matrix/scoring approach to assess different indications. Outside expertise is vital; you can't do this yourself.

Elizabeth Jeffards and Erin Newman from Alkahest further elaborated on this process of indication selection, and then moved on to talk about how to run trials. Their high level point was that the operation of trials becomes your whole company, determining everything about how you are seen and how you proceed. The two talked about the central matter of payer willingness to pay for your therapies – whether insurance giants, Medicare, and other entities will toe the line. This is a very important matter, at all stages of the process of figuring out which indication to pursue. They also emphasized the need to build a very specific target product profile, the exact cost and performance of your therapy, well in advance of any sort of data. Another vital issue is manufacturing: getting the timing right, given the lengthy duration of GMP manufacture, and the huge cost of that process. This is challenging and needs very careful management. Peter Milner of Retrotope talked about their orphan disease trials, and reinforced the points already made. Retrope uses deteurium stabilized lipids to treat neurodegenerative conditions in which lipid peroxidation is a serious concern. In talking about about the Retrotope clinical trials, he again pointed out that cost and time are very important in their choices of indications – one has to look for large effects achieved in quick trials.

The first day of the conference opened with a keynote by Nathaniel David of UNITY Biotechnologies. He surveyed the common approaches to research aimed at intervention in aging, that small changes between species biochemistry leads to large changes in species life span, and so forth. Regarding UNITY, he discussed their human data on the performance of senolytics for osteoarthritis and for degeneration of the retina, such as dry macular degeneration. They are in phase II for osteoarthritis, with data coming out late in 2020. For the eye, they are still working on phase 1 safety data, also coming out late 2020. They are also in the earlier stages of developing senolytic treatments for lung and kidney diseases.

Following that, Joan Mannick of ResTORbio opened her presentation by lauding mTORC1 as a target, pointing to the large body of research in short-lived species. Following failure on their phase III trial for reducing influenza incidence, they are now focusing on neurodegenerative disease, particularly Parkinson's disease. They believe that raised autophagy via mTORC1 inhibition may help with aggregates in these conditions, and discussed some of the supporting evidence in animal models. Peter Fedichev of Gero presented on their AI program for small molecule drug repurposing and discovery. Based on their models of biochemical data from mice and humans, they divide aging into two overlapping processes that they call "aging" and "frailty" – these are names for portions of a data model, and don't necessarily map well to the common meanings of the words. Mice and humans have quite different proportions of "aging" versus "frailty." Gero has new data from lifespan and rejuvenation studies using compounds that they intend to repurpose: they have achieved some degree of slowing or reversal of aspects of aging via their drugs in mice. This essentially shows they can pick drugs that perform comparably to some of the historical efforts to achieve this sort of outcome, and can do so faster than was possible in the past.

Gino Cortopossi of UC Davis is working on new approaches to upregulate mitochondrial function. He discussed how his group carried out the discovery of drug candidates to try to target mitochondrial function, SHC, and MTORC1. This presentation was an exercise in thinking about how to test interventions of this nature, what sort of a path leads forward from there to the clinic, and how to organize a handoff from academia to Big Pharma. Hanadie Yousef of Juvena Therapeutics talked about their AI-driven program of mining the secretome of pluripotent cells. The Juvena staff are searching for secreted molecules that can be delivered as therapies to upregulate regenerative and stem cell capacity in old people. Their initial focus is on muscle regeneration in the context of age-related sarcopenia. In one of the more interesting presentations of the day, Matthias Hackl of TAmiRNA talked about biomarker development in the microRNA space. The TAmiRNA folk think that they should be able to use a blood sample to produce simultaneous measures of senescent cell burden in many different tissues via assessment of circulating miRNAs from the senescence-associated secretory phenotype (SASP): each tissue has a signature. They are not quite there yet, but this will be very useful if it works out. Dana Larocca of AgeX Therapeutics talked on the topic of exosomes. That AgeX is focused on production of useful cell lines via induced pluripotency gives them a good head start on the production of useful exosomes via harvesting cell cultures of those cell lines. They are presently engaged in the search for interesting exosomes that might form the basis for therapies that make adult stem cells more active.

Jay Sarkar of Turn.bio discussed their approach to transient epigenetic reprogramming in order to force cell function to become more youthful. They use mRNA for reprogramming, as they feel it gives them greater control, and precise control is very

important in their work – they must not push cells all the way into pluripotency, just shock them into better operations, and there is a fine line between those two outcomes. The Turn.bio staff are using in vitro cell data to suggest that they can affect various hallmarks of aging: changing certain cell properties and the overall transcription landscape. The approach doesn't lengthen telomeres, which is interesting; the most important thing it does, I would say, is to restore mitochondrial function. He showed data for chondrocytes, relevant to osteoarthritis, and the Turn.bio team are also trying a cell therapy approach on this front, to reprogram cells and then transplant them to see if they help. Additionally, they have worked in skin models to show reversal of aspects of aging there. Turn.bio is one of a growing number of companies working with Entos Pharmaceuticals to produce a non-toxic lipid nanoparticle vector to deliver their therapy in vivo. Given that, it isn't surprising that they are also working with Oisin Biotechnologies, who also use the Entos Pharmaceuticals platform, to see how senolytics plus reprogramming work in synergy.

Rich Allsop of University of Hawaii talked on the role of FOXO3, one of the few robustly longevity associated genes in humans, in influencing telomere shortening and inflammaging. It touches on the IGF-1 pathway, and a variant is associated with greater longevity in humans. These researchers think that the behavior of the variant is more to do with enhancer or promoter effects on gene expression, not functional differences in the protein, as the difference is in a non-coding region of the gene. There is some question as to whether inflammation causes a difference in the pace of telomere shortening, such as via faster replication of immune cells in response to inflammatory signaling, or whether the relationship functions in a different way. Michael Fossel of Telocyte discussed his view on telomeres, cellular senescence, and telomerase gene therapy. He argues that the data shows that you need to increase telomere length to a large degree in order to see reductions in cancer risk, meaning lots of telomerase, not just a little – too little and there will be more cancer. His company is presently looking for funding to run an Alzheimer's disease trial of telomerase gene therapy; they have everything planned, and just need the backing.

Steve Turner of InVivo Biosystems presented on a system that can be used to determine quickly, say in 3 months, whether or not a therapy will extend lifespan and healthspan. To achieve this result they use C. elegans and zebrafish, and assess omics results, with some degree of automation in their platform. In a related presentation, Gordon Lithgow of the Buck Institute outlined their work on small molecule discovery with a C. elegans platform. A fair number of varied approaches to cost-effectively use these short-lived species in conjunction with automation, omics, and AI are out there under development these days. Kristen Fortney of Bioage Labs talked on their AI-driven discovery in human aging omics data, in search of pathways that can be drugged. They take a holistic view of aging: don't study age-related diseases, study aging as a whole, look for important processes. Given pathways, they perform screening in vitro, then take drug candidates to a sizable vivarium of 3,000 mice (expanding to 12,000 all too soon), and test the outcome there. They outlined a few example targets and the data supporting their ongoing work, including approaches to reduce neuroinflammation. Andrea Maier of the University of Melbourne talked at a high level on the development of potential aging-targeting repurposed drugs in Australia. This was a very nuts and bolts outline regarding how one plans and conducts human trials for specific age-related diseases. They were largely focused on lifestyle intervention, and are only now starting to think about drugs. Rounding out the first day, Wim von Schooten of Teneobio presented on the use of a CD38 inhibitor as a way to upregulate NAD+ levels and mitochondrial function. CD38 is somewhat connected to the proximate causes of NAD+ reduction in aging mitochondria, but it has other roles as well. It is also anti-inflammatory. CD38 is upregulated with age, in concert with NAD+ drop and inflammation rise, and the position in this presentation is that CD38 is causal of NAD+ decline.

The second day of the conference kicked off with a presentation by Sergio Ruiz on the topic of the Methuselah Fund and their progress to date in supporting new and important companies in the longevity industry. He gave a general overview on the state of investment in early stage companies in the field: what investors are looking for; how to transition from lab to clinic; the recent evolution of the longevity industry and the field of aging research. He noted that this is a huge opportunity for changing the human condition, not just a chance for a sizable return on investment. The team is presently working on raising their second fund. The first fund writes $50k-$500k checks, the second fund will be much larger and write $1m-$5m checks.

Ronald Kohanski of the National Institute on Aging gave the NIA/NIH perspective on rejuvenation and accelerated aging as therapeutic targets. They see the Interventions Testing Program and other programs as ways in which the NIA supports industry. He noted a range of ongoing work at the NIA that connects to the hallmarks of aging. They are starting to think about using omics data from the Interventions Testing Program and other studies to better understand what is taking place in aging-related pathways, as well as to develop ways to measure rejuvenation and aging. The presentation mostly dwelled on parabiosis and small molecules that slow aging as interventions to consider in this context. Nir Barzilai of the Albert Einstein College of Medicine followed to talk about the challenges inherent in making therapies to target aging or age-related diseases. The first problem is that animal models are not great, there is too much failure in translation to human medicine. Then there is the issue of payers (insurance companies, medicare, and so on) that don't want to pay for interventions that slow aging, which is related to the challenge of there being no FDA-approved indication for aging. The lack of an indication is largely why payers will not pay, even if therapies could be approved in some useful way. He mixed this in with his usual talk about centenarians and data on their health habits, genetics, and so forth.

Kevin Perrott of OpenCures presented on collecting data from people who are trying interventions themselves, self-experimenters, to try to reduce the time taken to develop new therapies. He is conducting proteomic analysis of blood samples from people in the self-experimentation community to measure outcomes, and the OpenCures team are also carrying out volunteer studies of supplement-regulated compounds, somewhat similar to phase 1 trials in organization, with proteomic measurements to assess effects. Julie Andersen of the Buck Institute talked about cellular senescence as a driver of Alzheimer's disease – something I would like to see a lot more work on, given the potential for meaningful benefits to patients. She noted the evidence for senescent glial cells, such as astrocytes, to contribute to neurodegenerative pathology. It is now thought possible for post-mitotic neurons to undergo senescence as well, contrary to earlier dogma. That might present a challenge, but equally obvious issues with cognitive function haven't manifested yet in animal studies of senolytics. She presented in vitro evidence for amyloid-β to cause senescence in brain cells, and suggested that the spread of senescence via the SASP occurs without amyloid-β in the later stages of the condition. The initial presence of amyloid-β is required, but not thereafter, and might be why removing amyloid-β doesn't help once this process is underway.

Richard Marshak of Torcept Therapeutics undertook a discussion on how to go about rational drug development with aging as the target. The company conducts drug discovery of mTORC1 inhibitors, and he talked about their pipeline and evidence. There is still skepticism from Big Pharma regarding the whole of the longevity industry: there are no clear endpoints; the technical and regulatory risk is far greater than Big Pharma entities are usually prepared to engage with; and the expense of testing against aging as a target is believed to be high. Once again this included a discussion of payers versus regulators, and what these two groups are looking for. Payers are interested in extending healthy longevity, it is worth bearing this in mind – there are strong economic incentives here that may help to overcome other issues. The development of endpoints for interventions in aging is important, since we can't use aging itself right now. Yet surrogate outcomes (measurements of biomarkers rather than patient outcomes) are not popular with anybody in the regulatory system at this time.

Marco Quarta at Rubedo Life Sciences presented on their small molecule discovery of senolytic and anticancer compounds. They are at the preclinical stage and would like to start looking at other cell changes that occur with age as well, such as loss of stem cell function. They are claiming a 60-70% clearance of senescent cells in multiple tissues via their lead senolytic, which is larger than most of the published literature to date – but it is hard to say how this compares with the state of the art in the various companies working on new senolytics. A range of other mouse model data on toxicity, safety, and effectiveness was presented. Andy Schile of Jackson Laboratory gave a plug for their aged mice, a source for studies. He surrounded that with examples of some of their studies of mice at different ages, presenting data on their usefulness in various models of age-related disease and dysfunction. Pan Zheng from the University of Maryland Baltimore talked about the role of CD24 in the inflammatory response to tissue damage, such as in the context of graft versus host disease, for example. This research group is attempting to influence the CD24 pathway to reduce inflammation in bone marrow grafts, HIV patients, and during immunotherapy. They have a CD24 fusion protein that works via affecting immune checkpoints to dampen the response.

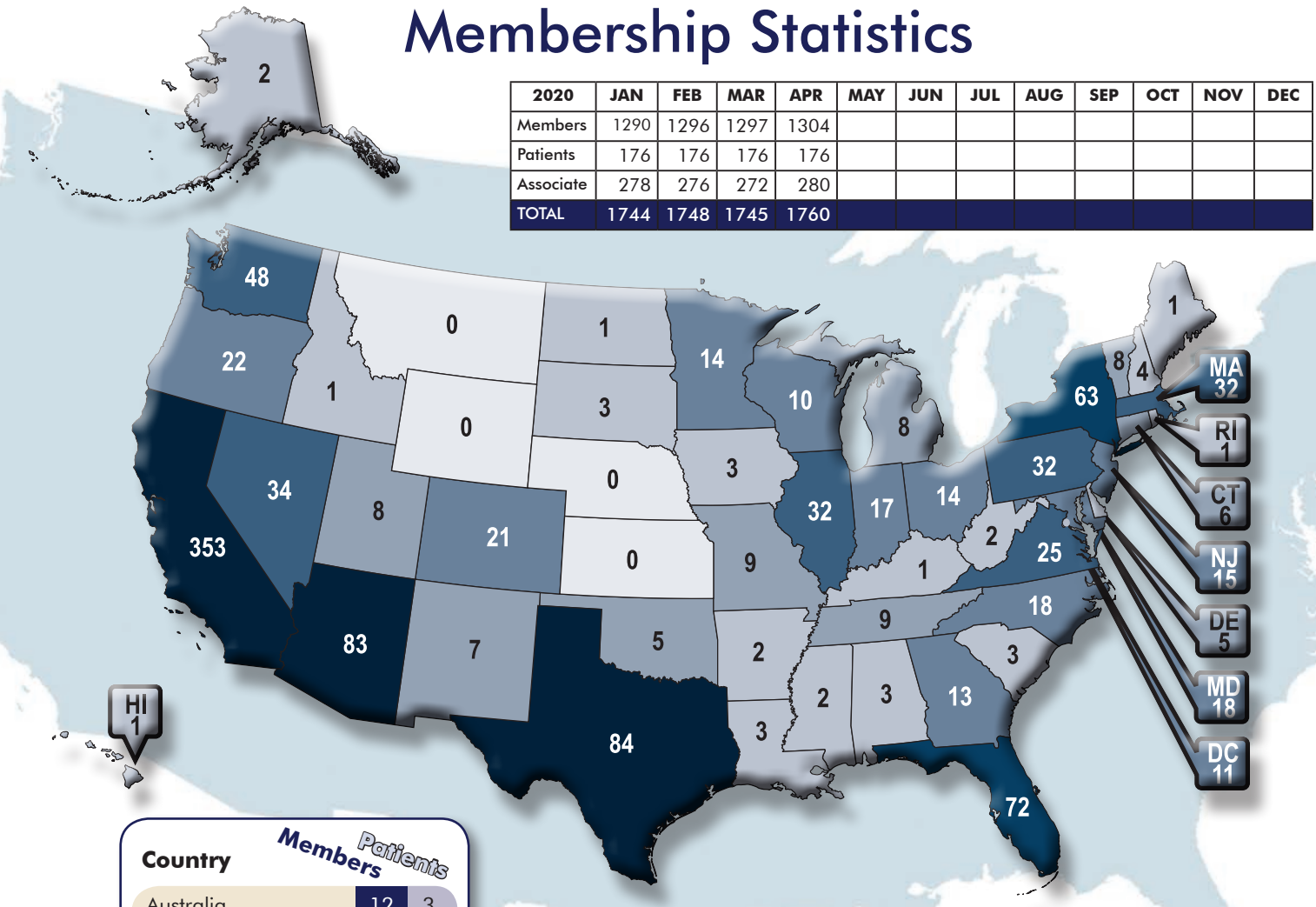
Jean-Marc Brondello of ISERM discussed cellular senescence as a cause of osteoarthritis, with a focus on the details of the manifestations of the condition and how senescence contributes to these issues. This team is processing omics data to identify possible new senotherapeutics that might address the issue. John Lewis of Oisin Biotechnologies gave the usual presentation on the Entos Pharmaceuticals lipid nanoparticle platform and its application as a senolytic therapy when delivering a suicide gene therapy triggered by expression of p16 or p53. An important point emphasized here is the exceptional safety profile of these nanoparticles – massive doses can be supplied to mice and other mammals with no signs of toxicity. Andrei Gudkov at Genome Protection discussed retrotransposons and their role in aging. Of particular interest is that retrotransposon activity drives chronic inflammation via cellular senescence. This team is developing therapies to try to ameliorate these issues. He presented an interesting view of aging as a species-specific cliff of mortality, and argues that DNA damage (i.e. retrotransposon activity) is the cause of the cliff, via production of chronic inflammation at a time dictated by loss of suppression of retrotransposon activity. Genome Protection studies retrotransposons in dogs, as breed variations in lifespan may be largely caused by retrotransposon based changes – the differences in genetics between dog breeds tend to cluster in locations connected to retrotranspon activity. Lastly, Lewis Gruber from SIWA Therapeutics presented on their program focused on a senolytic monoclonal antibody. They originally started out by targeting oxidative stress and glycolysis: these aspects of cell dysfunction have a common advanced glycation endproduct surface marker for a monoclonal antibody to bind to, given that binding, immune cells then destroy the errant cell. He pointed out that these marked cells are largely senescent, but others might only be dysfunctional. That can include cancerous cells.

All in all it was an interesting event, a good chance to catch up with existing members of the community and meet some new faces. If one has an interest in joining the longevity industry in some way, Longevity Therapeutics should be on the list of conferences to attend, along with Undoing Aging in Berlin, Ending Age-Related Diseases in New York, and Longevity Leaders and the Longevity Week events in London.

Send email to Reason at Fight Aging!: reason@fightaging.org

Membership Statistics

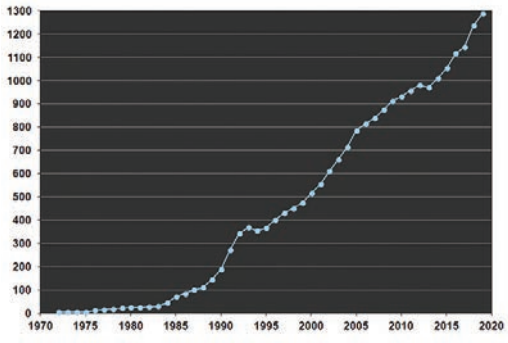
2020	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Members	1290	1296	1297	1304								
Patients	176	176	176	176								
Associate	278	276	272	280								
TOTAL	1744	1748	1745	1760								



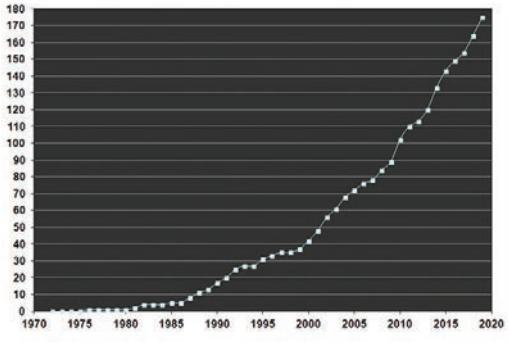
International Members & Patients

Country	Members	Patients
Australia	12	3
Austria	1	0
Belgium	1	0
Brazil	1	0
Bulgaria	1	0
Canada	62	4
China	0	1
Finland	1	0
France	0	1
Germany	20	0
Hong Kong	2	0
Hungary	1	0
Israel	1	1
Italy	2	0
Japan	4	0
Luxembourg	1	0
Mexico	5	0
Monaco	1	0
Netherlands	1	0
New Zealand	1	0
Norway	2	0
Portugal	4	1
Puerto Rico	1	0
South Korea	1	0
Spain	5	1
Taiwan	1	0
Thailand	2	1
United Kingdom	42	3
TOTAL	175	16

- 0 Members
- 1–4 Members
- 5–9 Members
- 10–24 Members
- 25–49 Members
- 50–74 Members
- 75+ Members



Number of Alcor members



Number of Alcor patients

Alcor Associate Membership

Supporters of Alcor who are not yet ready to make cryopreservation arrangements can become an Associate Member for $5/month (or $15/quarter or $60 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**
- **Access to local Alcor meetings and training events**

ALCOR
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To become an Associate Member send a check or money order ($5/month or $15/quarter or $60 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 cryo-preserved 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.

Revival Update

Scientific Developments Supporting Revival Technologies

Reported by R. Michael Perry, Ph.D.

A Deep Learning Approach to Antibiotic Discovery

Jonathan M. Stokes, Kevin Yang, Kyle Swanson, Wengong Jin, Andres Cubillos-Ruiz, Nina M. Donghia, Craig R. MacNair, Shawn French, Lindsey A. Carfrae, Zohar Bloom-Ackerman, Victoria M. Tran, Anush Chiappino-Pepe, Ahmed H. Badran, Ian W. Andrews, Emma J. Chory, George M. Church, Eric D. Brown, Tommi S. Jaakkola, Regina Barzilay, James J. Collins

Cell, 20 Feb 2020; 180(4): 688-702, https://www.cell.com/cell/fulltext/S0092-8674(20)30102-1?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS0092867420301021%3Fshowall%3Dtrue, accessed 2 Mar. 2020.

Summary

Due to the rapid emergence of antibiotic-resistant bacteria, there is a growing need to discover new antibiotics. To address this challenge, we trained a deep neural network capable of predicting molecules with antibacterial activity. We performed predictions on multiple chemical libraries and discovered a molecule from the Drug Repurposing Hub – halicin – that is structurally divergent from conventional antibiotics and displays bactericidal activity against a wide phylogenetic spectrum of pathogens including Mycobacterium tuberculosis and carbapenem-resistant Enterobacteriaceae. Halicin also effectively treated Clostridioides difficile and pan-resistant Acinetobacter baumannii infections in murine models. Additionally, from a discrete set of 23 empirically tested predictions from >107 million molecules curated from the ZINC15 database, our model identified eight antibacterial compounds that are structurally distant from known antibiotics. This work highlights the utility of deep learning approaches to expand our antibiotic arsenal through the discovery of structurally distinct antibacterial molecules.

From: Artificial Intelligence Yields New Antibiotic: A Deep-Learning Model Identifies a Powerful New Drug that Can Kill Many Species of Antibiotic-Resistant Bacteria by Anne Trafton, MIT News, 20 Feb. 2020, http://news.mit.edu/2020/artificial-intelligence-identifies-new-antibiotic-0220, accessed 2 Mar. 2020.

Using a machine-learning algorithm, MIT researchers have identified a powerful new antibiotic compound. In laboratory tests, the drug killed many of the world's most problematic disease-causing bacteria, including some strains that are resistant to all known antibiotics. It also cleared infections in two different mouse models.

The computer model, which can screen more than a hundred million chemical compounds in a matter of days, is designed to pick out potential antibiotics that kill bacteria using different mechanisms than those of existing drugs.

"We wanted to develop a platform that would allow us to harness the power of artificial intelligence to usher in a new age of antibiotic drug discovery," says James Collins, the Termeer Professor of Medical Engineering and Science in MIT's Institute for Medical Engineering and Science (IMES) and Department of Biological Engineering. "Our approach revealed this amazing molecule which is arguably one of the more powerful antibiotics that has been discovered."

In their new study, the researchers also identified several other promising antibiotic candidates, which they plan to test further. They believe the model could also be used to design new drugs, based on what it has learned about chemical structures that enable drugs to kill bacteria.

"The machine learning model can explore, in silico, large chemical spaces that can be prohibitively expensive for traditional experimental approaches," says Regina Barzilay, the Delta Electronics Professor of Electrical Engineering and Computer Science in MIT's Computer Science and Artificial Intelligence Laboratory (CSAIL).

Barzilay and Collins, who are faculty co-leads for MIT's Abdul Latif Jameel Clinic for Machine Learning in Health (J-Clinic), are the senior authors of the study, which appears today in Cell. The first author of the paper is Jonathan Stokes, a postdoc at MIT and the Broad Institute of MIT and Harvard.

Self-Contained Neuromusculoskeletal Arm Prostheses

Max Ortiz-Catalan, Ph.D., Enzo Mastinu, Ph.D., Paolo Sassu, M.D., Oskar Aszmann, M.D., and Rickard Brånemark, M.D., Ph.D.

N Engl J Med 30 Apr 2020; 382:1732-1738, https://www.nejm.org/doi/full/10.1056/NEJMoa1917537, accessed 7 May 2020.

Summary

We report the use of a bone-anchored, self-contained robotic arm with both sensory and motor components over 3 to 7 years in four patients after transhumeral amputation. The implant allowed for bidirectional communication between a prosthetic hand and electrodes implanted in the nerves and muscles of the upper arm and was anchored to the humerus through osseointegration, the process in which bone cells attach to an artificial surface without formation of fibrous tissue. Use of the device did not require formal training and depended on the intuitive intent of the user to activate movement and sensory feedback from the prosthesis. Daily use resulted in increasing sensory acuity and effectiveness in work and other activities of daily life.

From: Mind-Controlled Prostheses that "Feel" for Real, Johanna Wilde, Chalmers Electrical Engineering News, 30 Apr 2020, https://www.chalmers.se/en/departments/e2/news/Pages/Mind-controlled-arm-prostheses-now-a-part-of-everyday-life.aspx, accessed 7 May 2020

For the first time, people with arm amputations can experience sensations of touch in a mind-controlled arm prosthesis that they use in everyday life. Three Swedish patients have lived, for several years, with this new technology – one of the world's most integrated interfaces between human and machine.

The advance is unique: the patients have used a mind-controlled prosthesis in their everyday life for up to seven years. For the last few years, they have also lived with a new function – sensations of touch in the prosthetic hand. This is a new concept for artificial limbs, which are called neuromusculoskeletal prostheses – as they are connected to the user's nerves, muscles, and skeleton.

A study in the New England Journal of Medicine reports that these prostheses have a natural function in the patients'daily lives.

The research was led by Max Ortiz Catalan, Associate Professor at Chalmers University of Technology, in collaboration with Sahlgrenska University Hospital, University of Gothenburg, and Integrum AB, all in Gothenburg, Sweden. Researchers at Medical University of Vienna in Austria and the Massachusetts Institute of Technology in the USA were also involved.

"Our study shows that a prosthetic hand attached to the bone and controlled by electrodes implanted in nerves and muscles can operate much more precisely than conventional prosthetic hands. We further improved the use of the prosthesis by integrating tactile sensory feedback that the patients use to mediate how hard to grab or squeeze an object. Over time, the ability of the patients to discern smaller changes in the intensity of sensations has improved," says Max Ortiz Catalan.

"The most important contribution of this study was to demonstrate that this new type of prosthesis is a clinically viable replacement for a lost arm. No matter how sophisticated a neural interface becomes, it can only deliver real benefit to patients if the connection between the patient and the prosthesis is safe and reliable in the long-term. Our results are the product of many years of work, and now we can finally present the first bionic arm prosthesis that can be reliably controlled using implanted electrodes, while also conveying sensations to the user in everyday life."

Attosecond Coherent Manipulation of Electrons in Tunneling Microscopy

M. Garg, K. Kern

Science 24 Jan 2020; 367:6476, 411-415, https://science.sciencemag.org/content/367/6476/411, accessed 7 May 2020.

Abstract

Nanoelectronic devices operating in the quantum regime require coherent manipulation and control over electrons at atomic length and time scales. We demonstrate coherent control over electrons in a tunnel junction of a scanning tunneling microscope by means of precise tuning of the carrier-envelope phase of two-cycle long (<6-femtosecond) optical pulses. We explore photon and field-driven tunneling, two different regimes of interaction of optical pulses with the tunnel junction, and demonstrate a transition from one regime to the other. Our results show that it is possible to induce, track, and control electronic current at atomic scales with subfemtosecond resolution, providing a route to develop petahertz coherent nanoelectronics and microscopy.

From: An Ultrafast Microscope for the Quantum World, (unattributed), Max-Planck-Gesellschaft, 24 Jan 2020, https://www.mpg.de/14389376/an-ultrafast-microscope-for-the-quantum-world, accessed 8 May 2020.

Processes taking place inside tiny electronic components or in molecules can now be filmed at a resolution of a few hundred attoseconds and down to the individual atom. … Manish Garg and Klaus Kern, researchers at the Max Planck Institute for Solid State Research in Stuttgart, have developed a microscope for the extremely fast processes that take place on the quantum scale. This microscope – a sort of HD camera for the quantum world – allows the precise tracking of electron movements down to the individual atom. …

To do this, the two physicists use ultrashort laser pulses in conjunction with a scanning tunneling microscope. The latter achieves atomic-scale resolution by scanning a surface with a tip that itself is ideally made up of just a single atom. Electrons tunnel between the tip and the surface – that is, they cross the intervening space even though they actually don't have enough energy to do so. As the effectiveness of this tunnelling process depends strongly on the distance the electrons have to travel, it

can be used to measure the space between the tip and a sample and therefore to depict even individual atoms and molecules on a surface. Until now, however, scanning tunneling microscopes did not achieve sufficient temporal resolution to track electrons.

"By combining a scanning tunnelling microscope with ultrafast pulses, it was easy to use the advantages of the two methods to compensate for their respective disadvantages," says Manish Garg. The researchers fire these extremely short pulses of light at the microscope tip – which is positioned with atomic precision – to trigger the tunnelling process. As a result, this high-speed camera for the quantum world can now also achieve HD resolution….

With the new technique, physicists can now measure exactly where electrons are at a specific time down to the individual atom and to an accuracy of a few hundred attoseconds. For example, this can be used in molecules that have had an electron catapulted out of them by a high-energy pulse of light, leading the remaining negative charge carriers to rearrange themselves and possibly causing the molecule to enter into a chemical reaction with another molecule. "Filming electrons in molecules live, and on their natural spatial and temporal scale, is vital in order to understand chemical reactivity, for example, and the conversion of light energy within charged particles, such as electrons or ions," says Klaus Kern, Director at the Max Planck Institute for Solid State Research.

Moreover, the technique not only allows researchers to track the path of electrons through the processors and chips of the future, but can also lead to a dramatic acceleration of the charge carriers: "In today's computers, electrons oscillate at a frequency of a billion hertz," says Klaus Kern. "Using ultrashort light pulses, it may be possible to increase their frequency to a trillion hertz." With this turbo booster for light waves, researchers could clear the way for light-wave electronics, which is millions of times faster than current computers. Therefore, the ultrafast microscope not only films processes in the quantum world, but also acts as the Director by interfering with these processes.

A 3D Human Brain–Like Tissue Model of Herpes-Induced Alzheimer's Disease

Dana M. Cairns, Nicolas Rouleau, Rachael N. Parker, Katherine G. Walsh, Lee Gehrke and David L. Kaplan

Science Advances, 06 May 2020, 6(19), eaay8828, https://advances.sciencemag.org/content/6/19/eaay8828, accessed 7 May 2020.

Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder that causes cognitive decline, memory loss, and inability to perform everyday functions. Hallmark features of AD – including generation of amyloid plaques, neurofibrillary tangles, gliosis, and inflammation in the brain – are well defined; however, the cause of the disease remains elusive. Growing evidence implicates pathogens in AD development, with herpes simplex virus type I (HSV-1) gaining increasing attention as a potential causative agent. Here, we describe a multidisciplinary approach to produce physiologically relevant human tissues to study AD using human-induced neural stem cells (hiNSCs) and HSV-1 infection in a 3D bioengineered brain model. We report a herpes-induced tissue model of AD that mimics human disease with multicellular amyloid plaque-like formations, gliosis, neuroinflammation, and decreased functionality, completely in the absence of any exogenous mediators of AD. This model will allow for future studies to identify potential downstream drug targets for treating this devastating disease.

From: 3D Brain Model offers New Evidence Linking Alzheimer's to Herpes Virus, Rich Haridy, New Atlas, 6 May 2020, https://newatlas.com/science/alzheimers-disease-herpes-virus-3d-brain-model-study-tufts/, accessed 7 May 2020.

For several decades scientists have been presenting research linking the onset of Alzheimer's disease with the presence of a herpes viral infection. While the dominant amyloid hypothesis has reigned over the majority of Alzheimer's research, a torrent of failed clinical trials testing drugs designed to directly attack the buildup of amyloid plaques led to some researchers reconsidering a number of alternative viral hypotheses.

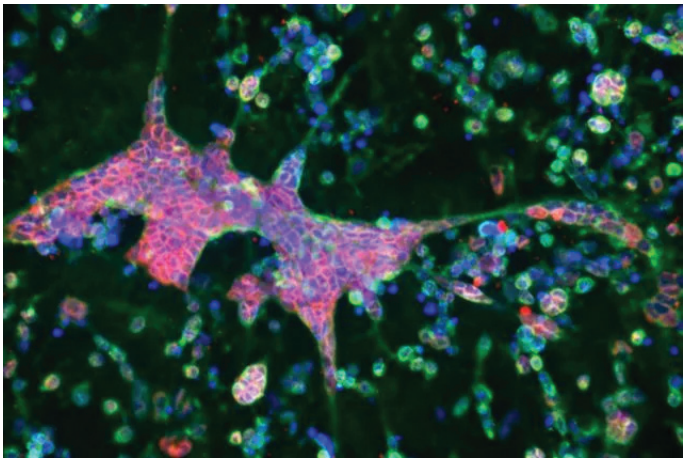
Direct causality between herpes simplex virus type I (HSV-1) and Alzheimer's disease is, unsurprisingly, difficult to establish. With over two-thirds of the world's population under the age of 50 estimated to carry HSV-1, it is clear that the virus could not be the sole cause of Alzheimer's. However, it may be that the virus, in combination with currently unknown genetic or environmental factors, triggers the pathogenic processes we later recognize as Alzheimer's disease.

To try and better understand this hypothetical causal process, the Tufts researchers constructed a novel 3D brain model composed of donut-shaped sponge-like materials, populated with neural stem cells that can be guided into neurons, mimicking both white and grey brain matter.

"This is a model of Alzheimer's disease which is very different from what other studies have used," explains first author on the study, Dana Cairns. "Most other studies relied on using genetic mutations in the neurons to induce Alzheimer's disease-like phenotypes and ours does not, which is what really sets it apart. Our model using normal neurons allows us to show that herpes virus alone is sufficient to induce Alzheimer's disease phenotypes."

The research is the first human brain tissue model to demonstrate a HSV-1 infection mediating the development of pathogenic signs resembling sporadic Alzheimer's disease. The results are important because the model was absent of any specific mediators that would otherwise induce Alzheimer's-like signs, other than an infection with HSV-1.

"After just three days of herpes infection, we saw large and dense plaque formations of beta amyloid protein, as well as increased expression of some of the enzymes responsible for generating the plaques," says senior author, David Kaplan. "We observed neuron loss, neuroinflammation and depressed signaling between neurons – everything we observe in patients. Never before have so many facets of the disease been replicated in vitro."



The 3D brain tissue model shows amyloid beta fibrils (in red), and herpes-infected neurons (in green) Dana Cairns, Tufts University

Replay of Learned Neural Firing Sequences during Rest in Human Motor Cortex

Jean Baptiste Eichenlaub, Beata Jarosiewicz, Jad Saab, Brian Franco, Jessica Kelemen, Eric Halgren, Leigh R. Hochberg, Sydney S. Cash

Cell Reports 31(5), 5 May 2020, 107581, https://www.sciencedirect.com/science/article/pii/S2211124720305301, accessed 7 May 2020.

Summary

The offline "replay" of neural firing patterns underlying waking experience, previously observed in non-human animals, is thought to be a mechanism for memory consolidation. Here, we test for replay in the human brain by recording spiking activity from the motor cortex of two participants who had intracortical microelectrode arrays placed chronically as part of a brain-computer interface pilot clinical trial. Participants took a nap before and after playing a neurally controlled sequence-copying game that consists of many repetitions of one "repeated" sequence sparsely interleaved with varying "control" sequences. Both participants performed repeated sequences more accurately than control sequences, consistent with learning. We compare the firing rate patterns that caused the cursor movements when performing each sequence to firing rate patterns throughout both rest periods. Correlations with repeated sequences increase more from pre- to post-task rest than do correlations with control sequences, providing direct evidence of learning-related replay in the human brain.

From: Landmark Research Watches Sleeping Brains Replay Waking Experiences, Rich Haridy, New Atlas, 5 May, 2020, https://newatlas.com/science/sleeping-brains-replay-waking-experiences-braingate-memory/, accessed 7 May 2020.

A landmark study, published in the journal *Cell Reports*, is offering the first direct evidence of a process by which our brains consolidate memories during sleep. The researchers witnessed neuronal firing patterns in humans during sleep that suggest we replay our waking experiences as we rest.

Scientists have for decades been studying exactly how the brain stores memories, and extensive animal tests have elucidated a process of sleep-based memory consolidation referred to as "offline replay." This is where a brain effectively replays neural firing patterns associated with waking events during sleep.

This process has been explicitly observed in animals but only indirectly studied in humans. The hurdle researchers have faced is that current brain imaging techniques, such as intracranial macro-electrode recordings or fMRI, do not have the spatial resolution to offer detailed data on human neural firing rate patterns.

In order to zoom in on offline replay neural activity in humans the scientists joined forces with an ongoing research project called BrainGate. In development for well over a decade, BrainGate involves implanting tiny microelectrode arrays into the brains of subjects with profound spinal injury. The goal of the research is to develop a brain-computer interface that helps those with severe motor disabilities control computers and other devices.

"There aren't a lot of scenarios in which a person would have a multi-electrode array placed in their brain, where the electrodes are tiny enough to be able to detect the firing activity of individual neurons," explains Beata Jarosiewicz, co-first author on the new study.

For the new research, two BrainGate subjects with tetraplegia were recruited. Baseline neural activity was recorded through an initial nap, then both subjects were tasked with playing a color sequence copying test modeled on the classic 80s video game, Simon. Of course, instead of physically directing a cursor, the subjects imagined moving the cursor in a desired sequence.

After playing the game, the subjects rested again allowing the researchers a detailed insight into their resting neural firing patterns. The study reports the subjects' brains repeating the same game-playing neuronal firing patterns while they rested.

"This is the first piece of direct evidence that in humans, we also see replay during rest following learning that might help to consolidate those memories," says Jarosiewicz. "All the replay-related memory consolidation mechanisms that we've studied in animals for all these decades might actually generalize to humans as well."

A Roadmap to Revival

Successful revival 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 is a list of landmark papers and books that reflect ongoing progress towards the revival 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.

Gregory M. Fahy, "**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(1) (January 1994):16-31 (Part I) & *Cryonics* 15(2) (April 1994):20-32 (Part II).

Ralph C. Merkle, "**Cryonics, Cryptography, and Maximum Likelihood Estimation**," First Extropy Institute Conference, Sunnyvale CA, 1994, updated version at http://www.merkle.com/cryo/cryptoCryo.html.

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, 685-805.

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

Robert A. Freitas Jr., "**The Alzheimer Protocols: A Nanorobotic Cure for Alzheimer's Disease and Related Neurodegenerative Conditions**," *IMM Report* No. 48, June 2016.

Ralph C Merkle, "**Revival of Alcor Patients**," *Cryonics*, 39(4) & 39(5) (May-June & July-August 2018): 10-19, 10-15.

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 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.

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- 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 ($5/month or $15/quarter or $60 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

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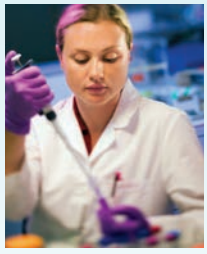

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