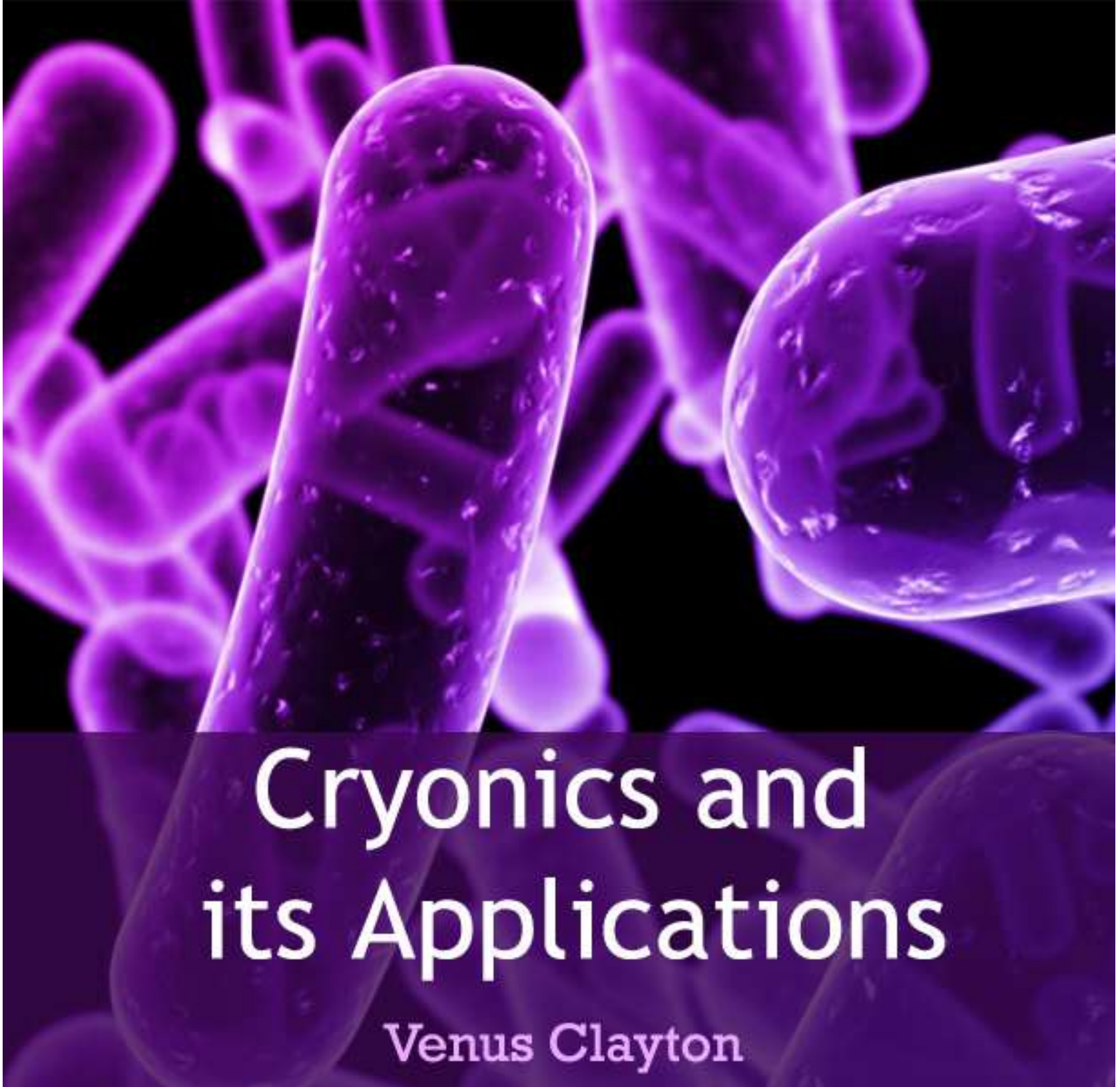

Cryonics and its Applications

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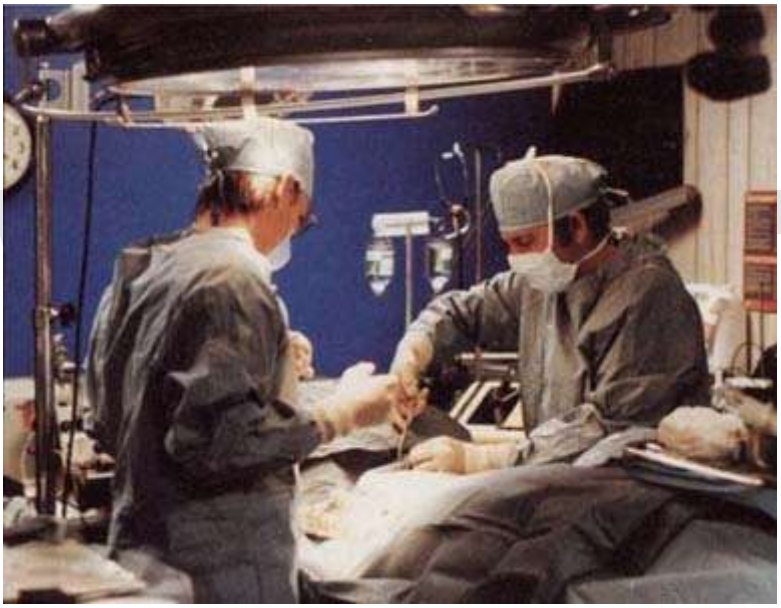
Cryonics



Technicians prepare a patient for cryopreservation.

Cryonics (from Greek *kryos*- meaning *icy cold*) is the low-temperature preservation of humans and animals who can no longer be sustained by contemporary medicine, with the hope that healing and resuscitation may be possible in the future. Cryopreservation of people or large animals is not reversible with current technology. The stated rationale for cryonics is that people who are considered dead by current legal or medical definitions may not necessarily be dead according to the more stringent information-theoretic definition of death. It is proposed that cryopreserved people might someday be recovered by using highly advanced future technology.

The future repair technologies assumed by cryonics are still hypothetical and not widely known or recognized. Responding to skepticism from scientists such as Steve Jones, an open letter supporting cryonics was written and signed by currently 62 scientists. As of 2010, only around 200 people have undergone the procedure since it was first proposed in 1962. In the United States, cryonics can only be legally performed on humans after they have been pronounced legally dead.

Cryonics procedures ideally begin within minutes of cardiac arrest, and use cryoprotectants to prevent ice formation during cryopreservation. However, the idea of cryonics also includes preservation of people after longer post-mortem delays because of the possibility that brain structures encoding memory and personality may still persist or be inferable. Whether sufficient brain information still exists for cryonics to work under some preservation conditions may be intrinsically unprovable by present knowledge. Therefore, most proponents of cryonics see it as an intervention with prospects for success that vary widely depending on circumstances.

Premises of cryonics

A central premise of cryonics is that long-term memory, personality, and identity are stored in durable cell structures and patterns within the brain that do not require continuous brain activity to survive. This premise is generally accepted in medicine; it is known that under certain conditions the brain can stop functioning and still later recover with retention of long-term memory. Additional scientific premises of cryonics are that (1) brain structures encoding personality and long-term memory persist for some time after clinical death, (2) these structures are preserved by cryopreservation, and (3) future technologies that could restore encoded memories to functional expression in a healed person are theoretically possible.

Cryonics is controversial because the technologies of premise (3) are so advanced that premises (1) and (2) are considered irrelevant by most scientists. Whether biological traces of memory or personhood might persist after clinical death is obviously a question of interest. Similarly, outside of cryonics there is no interest in the question of whether memory encoding might survive cryopreservation because the question is regarded as meaningless until cryopreservation can be reversed. At present only cells, tissues, and some small organs can be reversibly cryopreserved. Medical science is primarily concerned with what is demonstrably achievable, not what is theoretically possible. There are therefore no established scientific specialties or journals directly concerned with the scientific questions posed by cryonics.

Cryonics advocates claim that it is possible to preserve the fine cell structures of the brain in which memory and identity reside with present technology. They say that demonstrably reversible preservation is not necessary to achieve the present-day goal of cryonics, which is preservation of brain information that encodes memory and personal identity. They believe that current cryonics procedures can preserve the anatomical basis of mind, and that this should be sufficient to prevent information-theoretic death until future repairs might be possible.

A moral premise of cryonics is that cryopreserving people when there is no other hope is the right thing to do, sometimes even under poor conditions that make the scientific premises of cryonics highly uncertain. Some cryonicists believe as a matter of principle that anyone who would ordinarily be regarded as dead should instead be made a "permanent patient" subject to whatever future advances might bring.

Obstacles to success

Preservation injury

Long-term cryopreservation can be achieved by cooling to near 77.15 Kelvin, the boiling point of liquid nitrogen. It is a common mistaken belief that cells will lyse (burst) due to the formation of ice crystals within the cell, since this only occurs if the freezing rate exceeds the osmotic loss of water to the extracellular space. However, damage from freezing can still be serious; ice may still form between cells, causing mechanical and chemical damage. Cryonics organizations use cryoprotectants to reduce this damage. Cryoprotectant solutions are circulated through blood vessels to remove and replace water inside cells with chemicals that prevent freezing. This can reduce damage greatly, but freezing of whole people still causes injuries that are not reversible with present technology.

When used at high concentrations, cryoprotectants stop ice formation completely. Cooling and solidification without freezing is called vitrification. The first cryoprotectant solutions able to vitrify at very slow cooling rates while still being compatible with tissue survival were developed in the late 1990s by cryobiologists Gregory Fahy and Brian Wowk for the purpose of banking transplantable organs. These solutions were adopted for use in cryonics by the Alcor Life Extension Foundation, for which they are believed to permit vitrification of some parts of the human body, especially the brain. This has allowed animal brains to be vitrified, warmed back up, and examined for ice damage using light and electron microscopy. No ice crystal damage was found. The Cryonics Institute also uses a vitrification solution developed by their staff cryobiologist, Dr. Yuri Pichugin, applying it principally to the brain.

Vitrification in cryonics is different than vitrification in mainstream cryobiology because vitrification in cryonics is not reversible with current technology. It is only structural vitrification. When successful it can prevent freezing injury in some body parts, but at the price of toxicity caused by cryoprotectant chemicals. The nature of this toxicity is still poorly understood. Cryonicists assume that toxicity is more subtle and repairable than obvious structural damage that would otherwise be caused by freezing. If, for example, toxicity is due to denatured proteins, those proteins could be repaired or replaced.

Ischemic injury

Ischemia means inadequate or absent blood circulation that deprives tissue of oxygen and nutrients. At least several minutes of ischemia is a typical part of cryonics because of the common legal requirement that cryonics procedures do not begin until after blood circulation stops. The heart must stop beating so that legal death can be declared. When there is advance notice of impending clinical death, it is sometimes possible to deploy a team of technicians to perform a “standby”. The team artificially restores blood circulation and breathing using techniques similar to CPR as soon as possible after the heart stops. The aim is to keep tissues alive after legal death by analogy to conventional medical procedures in which viable organs and tissues are obtained for transplant from

legally deceased donors. Legal death does not mean that all the cells of the body have died.

Often in cryonics the brain is without oxygen for many minutes at warm temperatures, or even hours if the heart stops unexpectedly. This causes ischemic injury to the brain and other tissues that makes resuscitation impossible by present medical technology. Cryonicists justify preservation under such conditions by noting recent advances that allow brain resuscitation after longer periods of ischemia than the traditional 4-to-6-minute limit, and persistence of brain structure and even some brain cell function after long periods of clinical death. They argue that definitions of death change as technology advances, and the early stages of what is called “death” today is actually a form of ischemic injury that will be reversible in the future. They claim that personal survival during long periods of clinical death is determined by information theoretic criteria.

Revival

Those who believe that revival may someday be possible generally look toward advanced bioengineering, molecular nanotechnology, nanomedicine, or mind uploading as key technologies. Revival requires repairing damage from lack of oxygen, cryoprotectant toxicity, thermal stress (fracturing), freezing in tissues that do not successfully vitrify, and reversing the effects that caused the patient's death. In many cases extensive tissue regeneration will be necessary. Hypothetical revival scenarios generally envision repairs being performed by vast numbers of microscopic organisms or devices. These devices would restore healthy cell structure and chemistry at the molecular level, ideally before warming. More radically, mind transfer has also been suggested as a possible revival approach if and when technology is ever developed to scan the memory contents of a preserved brain.

It has sometimes been written that cryonics revival will be a last-in-first-out process. In this view, preservation methods will get progressively better until eventually they are demonstrably reversible, after which medicine will begin to reach back and revive people cryopreserved by more primitive methods. Revival of people cryopreserved by early cryonics technology may require centuries, if it is possible at all. People cryopreserved in the future, with better technology, may require less advanced technology to be revived because they will have been cryopreserved with better technology that caused less damage to tissue. The "last in, first out" view of cryonics has been criticized because the quality of cryopreservation depends on many factors other than the era in which cryopreservation takes place.

It has been claimed that if technologies for general molecular analysis and repair are ever developed, then theoretically any damaged body could be “revived”. Survival would then depend on whether preserved brain information was sufficient to permit restoration of all or part of the personal identity of the original person, with amnesia being the final dividing line between success and failure.

Neuropreservation

Neuropreservation is cryopreservation of the brain, often within the head, with surgical removal and disposal (usually cremation) of the rest of the body. Neuropreservation, sometimes called “neuro,” is one of two distinct preservation options in cryonics, the other being "whole body" preservation.

Neuropreservation is motivated by the brain's role as the primary repository of memory and personal identity. (For instance, spinal cord injury victims, organ transplant patients, and amputees retain their personal identity.) It is also motivated by the belief that reversing any type of cryonic preservation is so difficult and complex that any future technology capable of it must by its nature be capable of generalized tissue regeneration, including growth of a new body around a repaired brain. Some suggested revival scenarios for whole body patients even involve discarding the original body and regenerating a new body because tissues are so badly damaged by the preservation process. These considerations, along with lower costs, easier transportation in emergencies, and the specific focus on brain preservation quality, have motivated many cryonicists to choose neuropreservation.

The advantages and disadvantages of neuropreservation are often debated among cryonics advocates. Critics of neuropreservation note that the body is a record of much life experience, including learned motor skills (muscle memory). While few cryonicists doubt that a revived neuro patient would be the same person, there are wider questions about how a regenerated body might feel different from the original. Partly for these reasons (as well as for better public relations), the Cryonics Institute preserves only whole bodies. Some proponents of neuropreservation agree with these concerns, but still feel that lower costs and better brain preservation justify concentrating preservation efforts on the brain. About two-thirds of the patients stored at Alcor are neuropreservation patients. Although the American Cryonics Society no longer offers the neuropreservation option, about half of their patients are "neuros".

Financial issues

Costs of cryonics vary greatly, ranging from the basic fee of $10,000 for neuro (head or brain only) cryopreservation at the European cryonics company KrioRus, to more than $200,000 for whole body cryopreservation by Alcor with overseas and last-minute fees. Alcor's neuropreservation (just the head) is priced at 80,000. There is an extra $500 annual membership fee during life by Alcor. After payment of an initiaton fee, ACS full members pay an annual fee of $300 currently. To some extent these cost differences reflect differences in how fees are quoted. The Cryonics Institute fee does not include “standby” (a team waits for death to occur and begins procedures at bedside), transportation costs, or funeral director expenses outside of Michigan, which must be purchased as extras. CI Members wanting Standby and Transport from cryonics professionals can contract for additional payment to the Florida-based company Suspended Animation, Inc.

While cryonics is sometimes suspected of being greatly profitable, the high expenses of doing cryonics are well documented. The expenses are comparable to major transplant surgeries. The two most expensive things are standby expenses (a team of 5+ people needs to be hired for up to several weeks) and the money that must be set aside to generate interest to pay for storage of the patient in liquid nitrogen in perpetuity (especially for whole body patients).

The most common method of paying for cryonics is life insurance, which spreads the cost over many years. Cryonics advocates are quick to point out that such insurance is especially affordable for young people. Cryonics providers claim that even the most expensive cryonics plans are “affordable for the vast majority” of people in the industrialized world who really want it and plan for it in advance. With the advent of low-cost cryonics provided by companies such as KrioRus (so far in Europe only) cryonics becomes feasible even for last-minute cases.

Philosophical and ethical considerations

Cryonics is based on a view of dying as a process that can be stopped in the minutes, and perhaps hours, following clinical death. If death is not an event that happens suddenly when the heart stops, this raises philosophical questions about what exactly death is. In 2005 an ethics debate in the medical journal, Critical Care, noted “…few if any patients pronounced dead by today’s physicians are in fact truly dead by any scientifically rigorous criteria.” Cryonics proponent Thomas Donaldson has argued that “death” based on cardiac arrest or resuscitation failure is a purely social construction used to justify terminating care of dying patients. In this view, legal death and its aftermath are a form of euthanasia in which sick people are abandoned. Philosopher Max More suggested a distinction between death associated with circumstances and intention versus death that is absolutely irreversible. Absolutely irreversible death has also been called information-theoretic death, which implies destruction of the brain to such an extent that the original information content can no longer be recovered. Bioethicist James Hughes has written that increasing rights will accrue to cryonics patients as prospects for revival become clearer, noting that recovery of legally dead persons has precedent in the discovery of missing persons.

Ethical and theological opinions of cryonics tend to pivot on the issue of whether cryonics is regarded as interment or medicine. If cryonics is interment, then religious beliefs about death and afterlife may come into consideration. Resuscitation may be deemed impossible by those with religious beliefs because the soul is gone, and according to most religions only God can resurrect the dead. Cryonics advocates complain that theological dismissal of cryonics because it is interment is a circular argument because calling cryonics "interment" presumes a priori that cryonics cannot work. They believe future technical advances will validate their view that cryonics patients are recoverable, and therefore never really dead. If cryonics is regarded as medicine, with legal death as a mere enabling mechanism, then cryonics is a long-term coma with uncertain prognosis. It is continuing to care for sick people when others have given up.

Alcor has published a vigorous Christian defense of cryonics, including excerpts of a sermon by Lutheran Reverend Kay Glaesner. Noted Christian commentator John Warwick Montgomery has defended cryonics. In 1969, a Roman Catholic priest consecrated the cryonics capsule of Ann DeBlasio, one of the first cryonics patients. Many followers of Nikolai Fyodorovich Fyodorov see cryonics as an important step in the Common Cause project (reference: Fedorov seminar in Moscow, Russia on 25.11.2006).

At the request of the American Cryonics Society, in 1995, Philosopher Charles Tandy, Ph.D. authored a paper entitled "Cryonic-Hibernation in Light of the Bioethical Principles of Beauchamp and Childress." Dr. Tandy considered the four bioethical factors or principles articulated by philosophers Beauchamp and Childress as they apply to cryonics. These four principles are 1) respect for autonomy; 2) nonmaleficence; 3) beneficence; and 4) justice. Tandy concluded that in respect to all four principles "biomedical professionals have a strong (not weak) and actual (not prima facie, but binding) obligation to help insure cryonic-hibernation of the cryonics patient."

History

Benjamin Franklin, in a 1773 letter, expressed regret that he lived "in a century too little advanced, and too near the infancy of science" that he could not be preserved and revived to fulfil his "very ardent desire to see and observe the state of America a hundred years hence". In 1922 Alexander Yaroslavsky, member of Russian immortalists-biocosmists movement, wrote "Anabiosys Poem". However, the modern era of cryonics began in 1962 when Michigan college physics teacher Robert Ettinger proposed in a privately published book, *The Prospect of Immortality*, that freezing people may be a way to reach future medical technology. Even though freezing a person is apparently fatal, Ettinger argued that what appears to be fatal today may be reversible in the future. He applied the same argument to the process of dying itself, saying that the early stages of clinical death may be reversible in the future. Combining these two ideas, he suggested that freezing recently deceased people may be a way to save lives.

Slightly before Ettinger's book was complete, Evan Cooper (writing as Nathan Duhring) privately published a book called *Immortality: Physically, Scientifically, Now* that independently suggested the same idea. Cooper founded the Life Extension Society (LES) in 1964 to promote freezing people. Ettinger came to be credited as the originator of cryonics, perhaps because his book was republished by Doubleday in 1964 on recommendation of Isaac Asimov and Fred Pohl, and received more publicity. Ettinger also stayed with the movement longer. Nevertheless, cryonics historian R. Michael Perry has written "Evan Cooper deserves the principal credit for forming an organized cryonics movement."

Cooper's Life Extension Society became the seed tree for cryonics societies throughout the country where local cryonics advocates would get together as a result of contact through the LES mailing list. The actual word "cryonics" was invented by Karl Werner, then a student in the studio of William Katavolos at Pratt Institute in Brooklyn, NY, in

1965 in conjunction with the founding of the Cryonics Society of New York (CSNY) by Curtis Henderson and Saul Kent that same year. This was followed by the founding of the Cryonics Society of Michigan (CSM) and Cryonics Society of California (CSC) in 1966, and Bay Area Cryonics Society (BACS) in 1969 (renamed the American Cryonics Society, or ACS, in 1985). Neither CSNY nor CSC are currently in operation. CSM eventually became the Immortalist Society, a non-profit affiliate of the Cryonics Institute (CI), a cryonics service organization founded by Ettinger in 1976. CI now has more current cryonics patients than any other organization.

Although there was at least one earlier aborted case, it is generally accepted that the first person frozen with intent of future resuscitation was Dr. James Bedford, a 73-year-old psychology professor frozen under crude conditions by CSC on January 12, 1967. The case made the cover of a limited print run of *Life Magazine* before the presses were stopped to report the death of three astronauts in the *Apollo 1* fire instead. Bedford is still frozen today at Alcor.

Cryonics suffered a major setback in 1979 when it was discovered that nine bodies stored by the head of the CSC, Robert Nelson, in a cemetery in Chatsworth, California, had thawed due to depletion of funds by relatives, after being maintained for a year and a half at the personal expense of Nelson. Some of the bodies had apparently thawed years earlier without notification. Nelson was sued, and negative publicity slowed cryonics growth for years afterward. Of 17 documented cryonics cases between 1967 and 1973, only James Bedford remains cryopreserved today. Strict financial controls and requirements adopted in response to the Chatsworth scandal have resulted in the successful maintenance of almost all cryonics cases since that era.

The largest cryonics organization today, in terms of membership, was established as a nonprofit organization by Fred and Linda Chamberlain in 1972 as the Alcor Society for Solid State Hypothermia (ALCOR). In 1977, the name was changed to the Alcor Life Extension Foundation. In 1982, the Institute for Advanced Biological Studies (IABS), founded by Mike Darwin and Steve Bridge in Indiana, merged with Alcor. During the 1980s, Darwin worked with UCLA cardiothoracic surgery researcher Jerry Leaf at Alcor to develop a medical model for cryonics procedures. They pioneered the first consistent use of a cryonics procedure now known as a “standby”, in which a team waits to begin life support procedures at the bedside of a cryonics patient as soon as possible after the heart stops.

The oldest incorporated cryonics society still in existence is the American Cryonics Society (ACS). This tax-exempt 501(c)(3) membership organization was incorporated in 1969 as the Bay Area Cryonics Society (BACS) by a group of cryonics advocates that included two prominent Bay Area physicians, Dr. M. Coleman Harris and Dr. Grace Talbot. The first suspensions under BACS auspices were performed in 1974 by Trans Time, Inc., a for-profit company started by BACS members. BACS researcher Dr. Paul Segall, working with Jerry Leaf of CryoVita, developed a medical model to induce hypothermia shortly after pronouncement of death. Dr. Segall later went on to pioneer blood substitutes for use in both cryonic suspension and in mainstream medicine.

Cryonics received new support in the 1980s when MIT engineer Eric Drexler started publishing papers and books foreseeing the new field of molecular nanotechnology. His 1986 book, *Engines of Creation*, included an entire chapter on cryonics applications. Cryonics advocates saw the nascent field of nanotechnology as vindication of their long held view that molecular repair of injured tissue was theoretically possible. In the late 1980s Alcor member Dick Clair (who was dying of AIDS) sued for, and ultimately won for everyone, the right to be cryonically preserved in the State of California. Alcor's membership expanded tenfold within a decade, with a 30% annual growth rate between 1988 and 1992.

On July 24, 1988, a Ph.D. in computer science named Kevin Brown started an electronic mailing list called *CryoNet* that became a powerful tool of communication for the cryonics community. Numerous other mailing lists and web forums for discussing cryonics and the affairs of particular organizations have since appeared, but CryoNet remains a central point of contact for cryonicists.

Alcor was disrupted by political turmoil in 1993 when a group of activists left to start the CryoCare Foundation, and associated for-profit companies CryoSpan, Inc. (headed by Paul Wakfer) and BioPreservation, Inc. (headed by Mike Darwin). Darwin and collaborators made many technical advances during this time period, including a landmark study documenting high quality brain preservation by freezing with high concentrations of glycerol. CryoCare ceased operations in 1999 when they were unable to renew their service contract with BioPreservation. CryoCare's two patients stored at CryoSpan were transferred to Alcor. Several ACS patients stored at CryoSpan were transferred to CI.

There have been numerous, often transient, for-profit companies involved in cryonics. For-profit companies were often paired or affiliated with non-profit groups they served. Some of these companies, with non-profits they served in parentheses, were Cryonic Interment, Inc. (CSC), Cryo-Span Corporation (CSNY), Cryo-Care Equipment Corporation (CSC and CSNY), Manrise Corporation (Alcor), CryoVita, Inc. (Alcor), BioTransport, Inc. (Alcor), Trans Time, Inc. (BACS), Soma, Inc. (IABS), CryoSpan, Inc. (CryoCare and ACS), BioPreservation, Inc. (CryoCare and ACS), Kryos, Inc. (ACS), Suspended Animation, Inc. (CI, ACS, and Alcor). Trans Time and Suspended Animation are the only for-profit cryonics organizations that still exist.

The cryonics field seems to have largely consolidated around three non-profit groups, Alcor Life Extension Foundation, Cryonics Institute (CI), and the American Cryonics Society (ACS), all deriving significant income from bequests and donations. A newly formed non-profit group called the Cryonics Society was formally incorporated in 2006 but is devoted solely to promotion and public education of the cryonics concept.

As research in the 1990s revealed in greater detail the damaging effects of freezing, there was a trend to use higher concentrations of glycerol cryoprotectant to prevent freezing injury. In 2001 Alcor began using vitrification, a technology borrowed from mainstream organ preservation research, in an attempt to completely prevent ice formation during

cooling. Initially the technology could only be applied to the head when separated from the body. In 2005 Alcor began treating the whole body with their vitrification solution in a procedure called "neurovitrification with whole body cryoprotection". In the same year, the Cryonics Institute began treating the head of their whole body patients with their own vitrification solution.

The Cryonics Institute maintains 98 human patients as of 9 July 2010 (along with about 70 pets) at its Clinton Township, Michigan facility. About a fifth of the cryopreserved humans and a smaller portion of the pets came to the CI facility through contract with the American Cryonics Society (which has no storage facilities of its own). Alcor currently maintains 98 cryonics patients in Scottsdale, Arizona. There are support groups in Europe, Canada, the United Kingdom, and Australia. There is also a smaller cryonics company in Russia maintains 12 human patients and 5 pets called KrioRus, and plans for a facility in Australia. There are also plans being developed by renowned architect Stephen Valentine for a multi-acre futuristic high security facility called Timeship to be built in an undisclosed location in the United States, as well as for an underground facility in Switzerland.

Chapter- 2

Cryopreservation and Neuropreservation

Cryopreservation

Cryopreservation of plant shoots. Open tank of liquid nitrogen behind.

A tank of liquid nitrogen, used to supply a cryogenic freezer (for storing laboratory samples at a temperature of about −150 degrees Celsius).

Cryopreservation is a process where cells or whole tissues are preserved by cooling to low sub-zero temperatures, such as (typically) 77 K or −196 °C (the boiling point of liquid nitrogen). At these low temperatures, any biological activity, including the biochemical reactions that would lead to cell death, is effectively stopped. However, when cryoprotectant solutions are not used, the cells being preserved are often damaged due to freezing during the approach to low temperatures or warming to room temperature.

Temperature

Cryogenic storage at very low temperatures is presumed to provide an indefinite, if not near infinite, longevity to cells although the actual “shelf life” is rather difficult to prove. In experiments with dried seeds, researchers found that there was noticeable variability in deterioration when samples were kept at different ‘frozen’ temperatures–even ultra cold ones. Temperatures below the glass transition point (Tg) of polyol's water solutions (around minus 136°C) appear to be accepted as the range where biological activity very substantially slows down, and minus 196°C (liquid phase of liquid nitrogen) is the preferred temperature for the storage of important specimens. While fridges, deep freezers and extra cold deep freezers, all similar to domestic ones, are used for many items, generally the ultra cold of liquid nitrogen at -196°C is required for successful preservation of the more complex biological structures to virtually stop all biological activity.

Risks

Phenomena which can cause damage to cells during cryopreservation mainly occur during the freezing stage, and include: solution effects, extracellular ice formation, dehydration and intracellular ice formation. Many of these effects can be reduced by cryoprotectants.

When having reached the frozen stage, the preserved material is relatively safe from further damage. However, estimates based on the accumulation of radiation-induced DNA damage during cryogenic storage have suggested a maximum storage period of 1000 years.

Solution effects

As ice crystals grow in freezing water, solutes are excluded, causing them to become concentrated in the remaining liquid water. High concentrations of some solutes can be very damaging.

Extracellular ice formation

When tissues are cooled slowly, water migrates out of cells and ice forms in the extracellular space. Too much extracellular ice can cause mechanical damage to the cell membrane due to crushing.

Dehydration

The migration of water causing extracellular ice formation can also cause cellular dehydration. The associated stresses on the cell can cause damage directly.

Intracellular ice formation

While some organisms and tissues can tolerate some extracellular ice, any appreciable intracellular ice is almost always fatal to cells.

Main methods to prevent risks

The main techniques to prevent cryopreservation damages are a well established combination of *controlled rate* and *slow freezing* on one hand, and a newer flash-freezing process known as *vitrification* on the other.

Slow programmable freezing

Controlled-rate and slow freezing, also called *slow programmable freezing (SPF)*, is a set of well established techniques pioneered in the early 1970s which enabled the first human embryo frozen birth Louise Brown in 1978. Since then machines that freeze biological samples using programmable steps, or controlled rates, have been used all over the world for human, animal and cell biology – 'freezing down' a sample to better preserve it for eventual thawing, before it is deep frozen, or cryopreserved, in liquid nitrogen. Such machines are used for freezing oocyte, skin, blood products, embryo, sperm, stem cells and general tissue preservation in hospitals, veterinary practices and research labs around the world. As an example, estimates put the number of live births from frozen embryos 'slow frozen' at some 300,000 to 400,000 or 20% of the estimated 3 million IVF births.

Lethal intracellular freezing can be avoided if cooling is slow enough to permit sufficient water to leave the cell during progressive freezing of the extracellular fluid. That rate differs between cells of differing size and water permeability: a typical cooling rate around 1°C/minute is appropriate for many mammalian cells after treatment with cryoprotectants such as glycerol or dimethyl sulphoxide, but the rate is not a universal optimum.

Several independent studies have provided evidence that frozen embryos stored using slow-freezing techniques may in some ways be 'better' than fresh in IVF. The studies were presented at the American Society for Reproductive Medicine conference in San Francisco, US, 2008. The studies indicate that using frozen embryos rather than fresh embryos reduced the risk of stillbirth and premature delivery though the exact reasons are still being explored.

Vitrification

Researchers who have developed a new technique, vitrification, as of 2000 claim to provide the benefits of cryopreservation without damage due to ice crystal formation. In clinical cryopreservation, vitrification usually requires the addition of cryoprotectants prior to cooling. The cryoprotectants act like antifreeze: they lower the freezing temperature. They also increase the viscosity. Instead of crystallizing, the syrupy solution

turns into an amorphous ice—i.e., it vitrifies. Vitrification of water is promoted by rapid cooling, and can be achieved without cryoprotectants by an extremely rapid drop in temperature (megakelvins per second). The rate that is required to attain glassy state in pure water was considered to be impossible until 2005.

Two conditions usually required to allow vitrification are an increase in the viscosity and a depression of the freezing temperature. Many solutes do both, but larger molecules generally have larger effect, particularly on viscosity. Rapid cooling also promotes vitrification.

In established methods of cryopreservation, the solute must penetrate the cell membrane in order to achieve increased viscosity and depress freezing temperature inside the cell. Sugars do not readily permeate through the membrane. Those solutes that do, such as dimethyl sulfoxide, a common cryoprotectant, are often toxic in high concentration. One of the difficult compromises faced in vitrifying cryopreservation is limiting the damage produced by the cryoprotectant itself.

Freezable tissues

In general, cryopreservation is easier for thin samples and small clumps of individual cells, because these can be cooled more quickly and so require lower doses of toxic cryoprotectants. Therefore, the goal of cryopreserving human livers and hearts for storage and transplant is still some distance away.

Nevertheless, suitable combinations of cryoprotectants and regimes of cooling and rinsing during warming often allow the successful cryopreservation of biological materials, particularly cell suspensions or thin tissue samples. Examples include:

- Semen
- Blood
    - Special cells for transfusion
    - Stem cells. It is optimal in high concentration of synthetic serum, stepwise equilibration and slow cooling.
    - Umbilical cord blood
- Tissue samples like tumors and histological cross sections
- Eggs (oocytes)
- Embryos that are 2, 4 or 8 cells when frozen
- Ovarian tissue
- Plant seeds or shoots may be cryopreserved for conservation purposes.

In addition, efforts are underway to preserve humans cryogenically, known as cryonics. In such efforts either the brain within the head or the entire body may undergo the above process. Cryonics is in a different category from the aforementioned examples, however: while countless cryopreserved cells, vaccines, tissue and other biologial samples have been thawed and successfully used, this has not yet been the case at all for cryopreserved brains or bodies. At issue are the criteria for defining "success". Proponents of cryonics

claim that cryopreservation using present technology, particularly vitrification of the brain, may be sufficient to preserve people in an "information theoretic" sense so that they could be revived and made whole by hypothetical vastly advanced future technology.

Semen

Semen can be used successfully almost indefinitely after cryopreservation. The longest reported successful storage is 21 years. It can be used for sperm donation where the recipient wants the treatment in a different time or place, or for men undergoing a vasectomy to still have the option to have children.

Testicular tissue

Cryopreservation of immature testicular tissue is a developing method to avail reproduction to young boys who need to go through gonadotoxic therapies. Animal data look promising, since healthy offsprings have been obtained after transplantation of frozen testicular cell suspensions or tissue pieces. However, none of the fertility restoration options from frozen tissue, i.e. cell suspension transplantation, tissue grafting and in vitro maturation (IVM) have proved efficient and safe in humans as yet.

Oocytes

Human *Oocyte cryopreservation* is a new technology in which a woman's eggs (oocytes) are extracted, frozen and stored. Later, when she is ready to become pregnant, the eggs can be thawed, fertilized, and transferred to the uterus as embryos.

Embryos

Cryopreservation for embryos are used for *embryo storage*, e.g. when in vitro fertilization has resulted in more embryos than is currently needed.

Pregnancies have been reported from embryos stored for 16 years. Many studies have evaluated the children born from frozen embryos, or "frosties". The result has uniformly been positive with no increase in birth defects or development abnormalities.

From October 1, 2009 human embryos are allowed to be stored for 10 years in the UK, according to the Human Fertilisation and Embryology Act 2008.

World usage data is hard to come by but it was reported in a study of 23 countries that almost 42,000 frozen human embryo transfers were performed during 2001 in Europe.

A study of more than 11,000 cryopreserved human embryos showed no significant effect of storage time on postthaw survival for IVF or oocyte donation cycles, or for embryos frozen at the pronuclear or cleavage stages. In addition, the duration of storage had no significant effect on clinical pregnancy, miscarriage, implantation, or live birth rate,

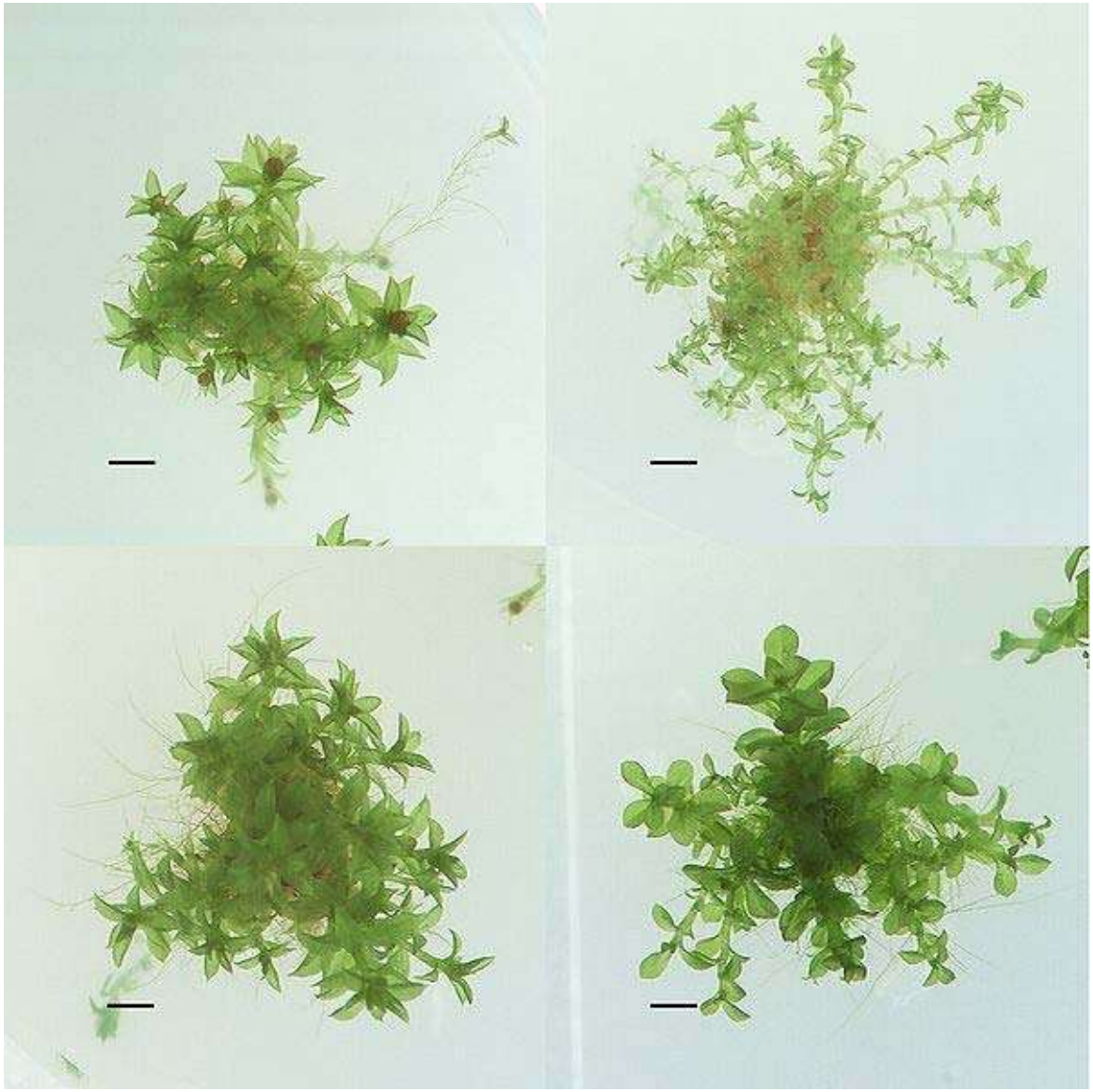
whether from IVF or oocyte donation cycles. Rather, oocyte age, survival proportion, and number of transferred embryos are predictors of pregnancy outcome.

Children born from vitrified blastocysts have significantly higher birthweight than those born from non-frozen blastocysts.

Ovarian tissue

Cryopreservation of ovarian tissue is of interest to women who want to preserve their reproductive function beyond the natural limit, or whose reproductive potential is threatened by cancer therapy, for example in hematologic malignancies or breast cancer. The procedure is to take a part of the ovary and carry out slow freezing before storing it in liquid nitrogen whilst therapy is undertaken. Tissue can then be thawed and implanted near the fallopian, either orthotopic (on the natural location) or heterotopic (on the abdominal wall), where it starts to produce new eggs, allowing normal conception to take place. The ovarian tissue may also be transplanted into mice that are immunocompromised (SCID mice) to avoid graft rejection, and tissue can be harvested later when mature follicles have developed.

Moss



Four different ecotypes of Physcomitrella patens stored at the IMSC.

Cryopreservation of whole moss plants, especially Physcomitrella patens, has been developed by Ralf Reski and coworkers and is performed at the International Moss Stock Center. This biobank collects, preserves, and distributes moss mutants and moss ecoptypes

Natural cryopreservation

Water bears (Tardigrada), microscopic multicellular organisms, can survive freezing at low temperatures by replacing most of their internal water with the sugar trehalose, preventing it from crystallization that otherwise damage cell membranes. Mixtures of solutes can achieve similar effects. Some solutes, including salts, have the disadvantage that they may be toxic at high concentrations. In addition to the Water bear, wood frogs can tolerate the freezing of their blood and other tissues. Urea is accumulated in tissues in preparation for overwintering, and liver glycogen is converted in large quantities to glucose in response to internal ice formation. Both urea and glucose act as "cryoprotectants" to limit the amount of ice that forms and to reduce osmotic shrinkage of cells. Frogs can survive many freeze/thaw events during winter if not more than about 65% of the total body water freezes. Research exploring the phenomenon of "Freezing frogs" has been primarily carried out by the Canadian researcher, Dr. Kenneth B. Storey.

Freeze tolerance, in which organisms survive the winter by freezing solid and ceasing life functions, is known in a few vertebrates: five species of frogs (*Rana sylvatica*, *Pseudacris triseriata*, *Hyla crucifer*, *Hyla versicolor*, *Hyla chrysoscelis*), one salamander (*Hynobius keyserlingi*), one snake (*Thamnophis sirtalis*) and three turtles (*Chrysemys picta*, *Terrapene carolina*, *Terrapene ornata*). Snapping turtle *Chelydra serpentina* and wall lizard *Podarcis muralis* also survive nominal freezing but it has not been established to be adaptive for overwintering. In the case of *Rana sylvatica* one cryopreservant is ordinary glucose, which rises in concentration by approximately 19 mmol/l when the frogs are slowly cooled;

History

One of the most important early workers on the theory of cryopreservation was James Lovelock of Gaia theory fame. He suggested that damage to red blood cells during freezing was due to osmotic stresses. Lovelock in early 1950s had also suggested that increasing salt concentrations in a cell as it dehydrates to lose water to the external ice might cause damages to the cell. Cryopreservation of tissue in recent times started with the freezing of fowl sperm, which in 1957 was cryopreserved by a team of scientists in the UK led by Christopher Polge. The process moved into the human world in the 1950s with pregnancies obtained after insemination of frozen sperm. However, the rapid immersion of the samples in liquid nitrogen did not, for certain of these samples–such as types of embryos, bone marrow and stem cells–produce the necessary viability to make them usable on thawing. Increased understanding of the mechanism of freezing injury to cells emphasised the importance of controlled or slow cooling to obtain maximum survival on thawing of the living cells. A controlled rate cooling process, allowing biological samples to equilibrate to optimal physical parameters osmotically in a cryoprotectant (a form of anti-freeze) before cooling in a predetermined, controlled way proved necessary. The ability of cryoprotectants, in the early cases glycerol, to protect cells from freezing injury was discovered accidentally. Freezing injury has two aspects–direct damage from the ice crystals and secondary damage caused by the increase in

concentration of solutes as progressively more ice is formed. In 1963 Peter Mazur, at Oak Ridge National Laboratory in the USA, showed that lethal intracellular freezing could be avoided if cooling was slow enough to permit sufficient water to leave the cell during progressive freezing of the extracellular fluid. That rate differs between cells of differing size and water permeability: a typical cooling rate around 1°C/minute is appropriate for many mammalian cells after treatment with cryoprotectants such as glycerol or dimethyl sulphoxide, but the rate is not a universal optimum.

Neuropreservation

Neuropreservation is cryopreservation of the human brain with the intention of future resuscitation and regrowth of a healthy body around the brain. Usually the brain is left within the head for physical protection, so the whole head is cryopreserved. Neuropreservation is a type of cryonics procedure and, like cryonics in general, is considered highly speculative and reliant on future technologies. A cryonics patient who undergoes neuropreservation is said to be a neuropatient.

Recently, the procedure is most often done for the sake of vitrification of the brain (neurovitrification), which has not yet been perfected on a full body level and is seen by some as a superior method of preservation, causing almost no tissue damage.

Future recovery prospects

Cryonics proponents (providers Alcor and the Cryonics Institute, along with the community of people signed up for or interested in the process) claim that extremely advanced future technologies will be required for successful cryonics, such as mature nanomedicine. It is said these technologies must necessarily be capable of tissue and organ regeneration, so neuropreservation is just as likely (or unlikely) to work as whole body cryopreservation. Neuropreservation is typically less expensive than whole body cryopreservation, and can potentially result in better brain preservation because the process can be optimized for the brain. Neuropreservations are easier to maintain, and none has been lost to thawing as of yet.

The hypothetical future recovery process is said to involve programming cells on the brain to regenerate a new body around the repaired brain inside a fluid life support environment, requiring cell-by-cell repair technology, as cryonics in general would. News media sometimes report that new bodies are expected from cloning, but some cryonics experts dismiss cloning, claiming that nothing as crude as nuclear transfer or transplants will ever have to be used in cryonics. They believe the methods used for recovery of neuropatients will be an extension of mainstream medical technologies that will someday be developed to regrow lost limbs and treat severe trauma.

Another, equally speculative technology for the revival of neuropatients, or cryonics patients generally, is mind transfer. Although philosophically more radical, transferring the information content of a cryopreserved brain into an artificial brain may be no more

or less feasible than re-growing a biological body, especially to a society with technology capable of reviving cryopreserved brain tissue.

Advantages

Several advantages to neuropreservation over whole body preservation have been put forth. These include lower costs on the part of the patient and for storage, greater transportability in case of disaster, ease in reaching the brain with cryoprotectants and thus a better chance that the brain is preserved, and quicker cooling which may also increase likelihood of future recovery. Aubrey de Grey has theorized that neuropatients will be revived after procedures have been perfected on whole body patients, and therefore have better chances for revival.

History

Neuropreservation was first proposed in 1965 by cryonics co-creator Evan Cooper, proposed again in a speculative scientific paper by gerontologist George M. Martin in 1971, and independently proposed yet again in 1974 by Mike Darwin, and Fred and Linda Chamberlain. The Chamberlains were the founders of the Alcor Life Extension Foundation. In 1976 Fred's father became the first of many neuropreservation patients at Alcor.

Prior to the year 2000, neuropreservation was performed by surgical separation of the body from the head (called cephalic isolation or "neuroseparation") at the end of cryoprotectant perfusion performed on the upper body via the ascending aorta. After that year, Alcor began performing cephalic isolation before cryoprotectant perfusion, in deep hypothermia, and then using the carotid and vetebral arteries directly for perfusion with cryoprotectants.

As of 2008, Alcor and KrioRus are the only cryonics organizations that offer neuropreservation. Other organizations, such as the other major provider, the Cryonics Institute, avoid it because they say it is bad for public relations. Alcor claims there are good technical justifications for neuropreservation, and that they will continue to offer it. Approximately three quarters of the cryonics patients stored at Alcor are neuropatients.

Chapter- 3

Freezable Tissues

Chemical brain preservation

Chemical Brain Preservation is the process of preparing the brain, or entire central nervous system for long term, high quality storage. Unlike cryopreservation, chemical techniques do not require freezing and storage at extremely low temperatures. There is currently research into the development of a surgical protocol that can reliably and demonstrably preserve a human brain's precise neural circuitry for long-term (>100 years) storage. If such a procedure were available it would give interested persons a means of avoiding death and reaching the distant future.

Technology

Such a procedure would most likely involve either vascular perfusion of a person with cryoprotective agents and long-term storage at close to liquid nitrogen temperature, or vascular perfusion of a person with chemical fixative agents followed by a plasticizing agent and room temperature storage. Both techniques have already been successfully demonstrated on small pieces of brain, but advances in the chemical formulations, perfusion apparatus, and surgical technique are necessary to preserve all of the neural circuits in a human brain in a way which can be verified.

Given the already advanced state of the existing techniques, it is likely that preservation of a whole brain could be demonstrated within a 5 year time frame if appropriate intellectual and monetary resources were devoted to the problem; unfortunately, a general lack of understanding of the potential for human brain preservation has severely curtailed such research. A Brain Preservation Technology Prize has been offered by the Brain Preservation Foundation for meeting certain goals. As of June 12, 2010 the prize is valued at $100,000

Rationale

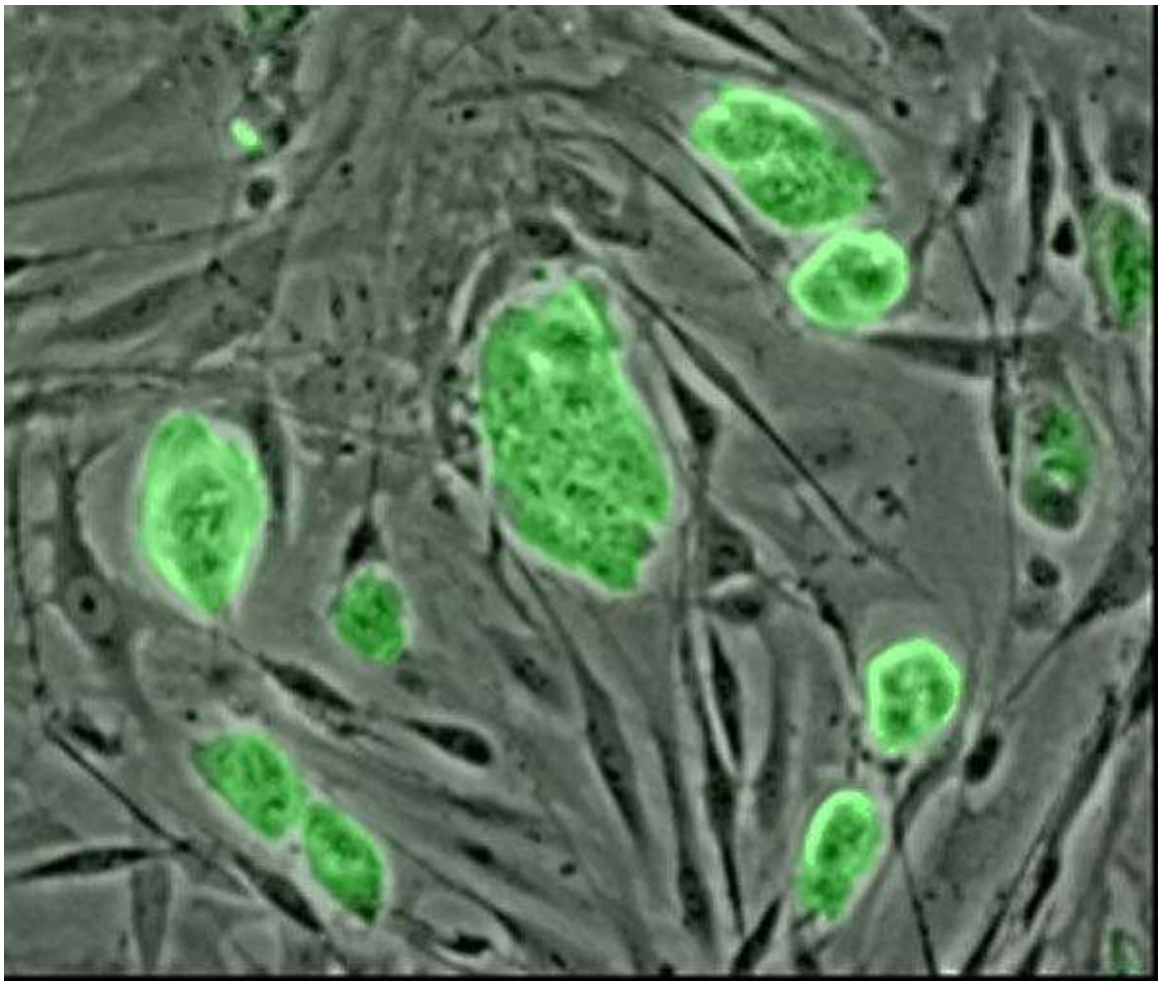
The idea of putting a person in 'suspended animation' so they can reach future medical technology has been a staple of science fiction for decades. It has also been practiced, in an ad hoc way, by an eclectic group called cryonicists. The purpose of a Brain

Preservation Technology Prize is to have an independent panel of scientists and medical doctors define a clear set of milestones which, if achieved, would warrant that such a preservation procedure be seriously considered as a viable medical alternative to death.

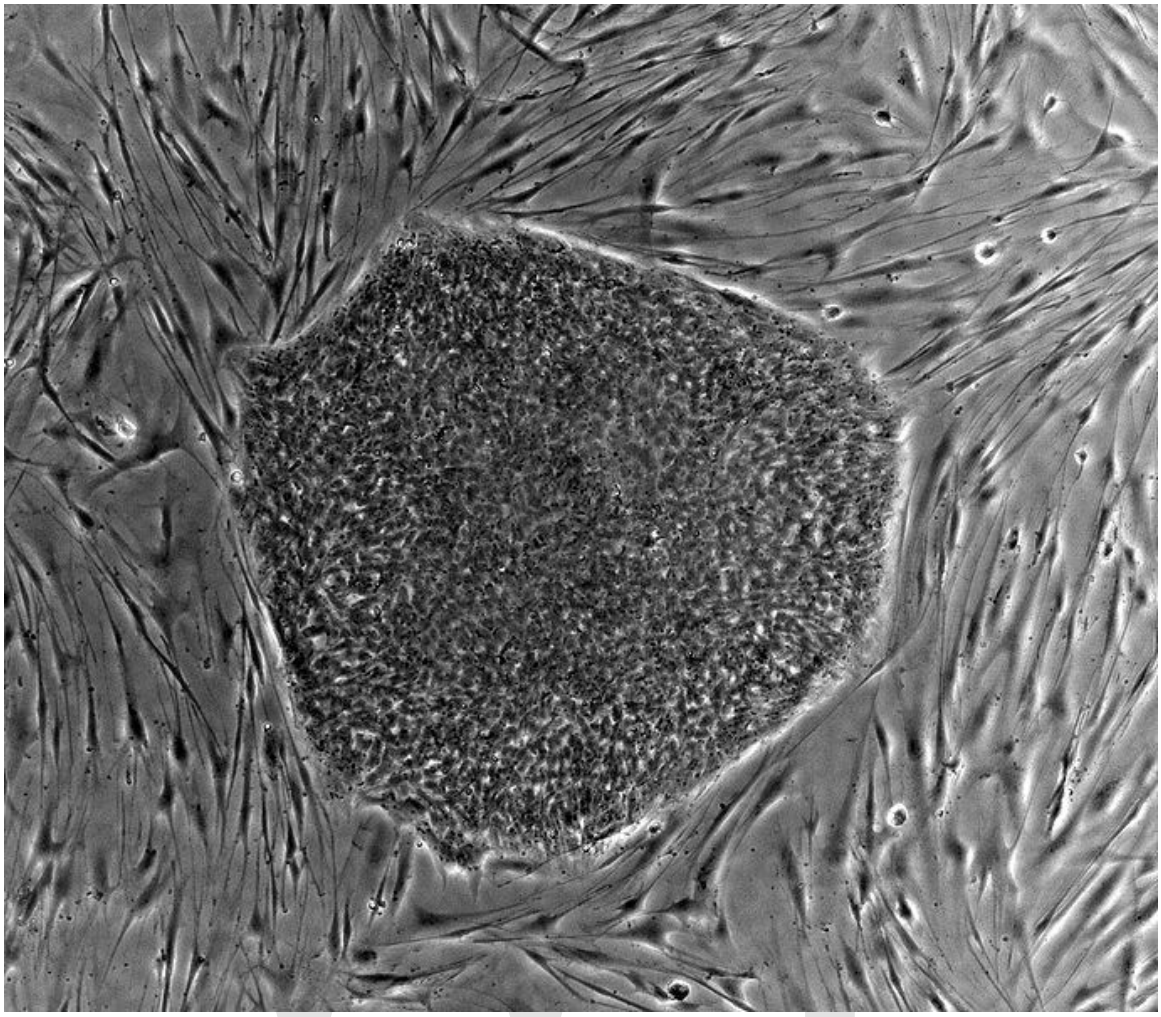
Background information

In 1962 Robert Ettinger put forward the radical idea that a person's brain and body might be preserved using the best available techniques so that the person could reach future medical technology of sufficient advancement to restore health. This idea, because it is in principle scientifically sound, initially attracted much interest from both scientists and laypersons and gave rise to the practice of low-temperature (cryonic) preservation. However, even the best cryonic techniques of the 1960s produced horrific damage to brain tissue as seen by light and electron microscopic examination. All reasonable scientists looking at this massive amount of damage to the neural connectivity of the brain rightly concluded that cryonic suspension using the existing techniques was hopeless. People selling cryopreservation to individuals were labeled as naive, or worse, quacks and charlatans, and the vast majority of scientists, especially cryobiologists, quickly distanced themselves from the entire enterprise.

Stem cell



Mouse embryonic stem cells with fluorescent marker



Human embryonic stem cell colony on mouse embryonic fibroblast feeder layer

Stem cells are cells found in all multi cellular organisms. They are characterized by the ability to renew themselves through mitotic cell division and differentiate into a diverse range of specialized cell types. Research in the stem cell field grew out of findings by Ernest A. McCulloch and James E. Till at the University of Toronto in the 1960s.

The two broad types of mammalian stem cells are: embryonic stem cells that are isolated from the inner cell mass of blastocysts, and adult stem cells that are found in adult tissues. In a developing embryo, stem cells can differentiate into all of the specialized embryonic tissues. In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenishing specialized cells, but also maintain the normal turnover of regenerative organs, such as blood, skin, or intestinal tissues.

Stem cells can now be grown and transformed into specialized cells with characteristics consistent with cells of various tissues such as muscles or nerves through cell culture. Highly plastic adult stem cells from a variety of sources, including umbilical cord blood and bone marrow, are routinely used in medical therapies. Embryonic cell lines and

autologous embryonic stem cells generated through therapeutic cloning have also been proposed as promising candidates for future therapies.

Properties

The classical definition of a stem cell requires that it possess two properties:

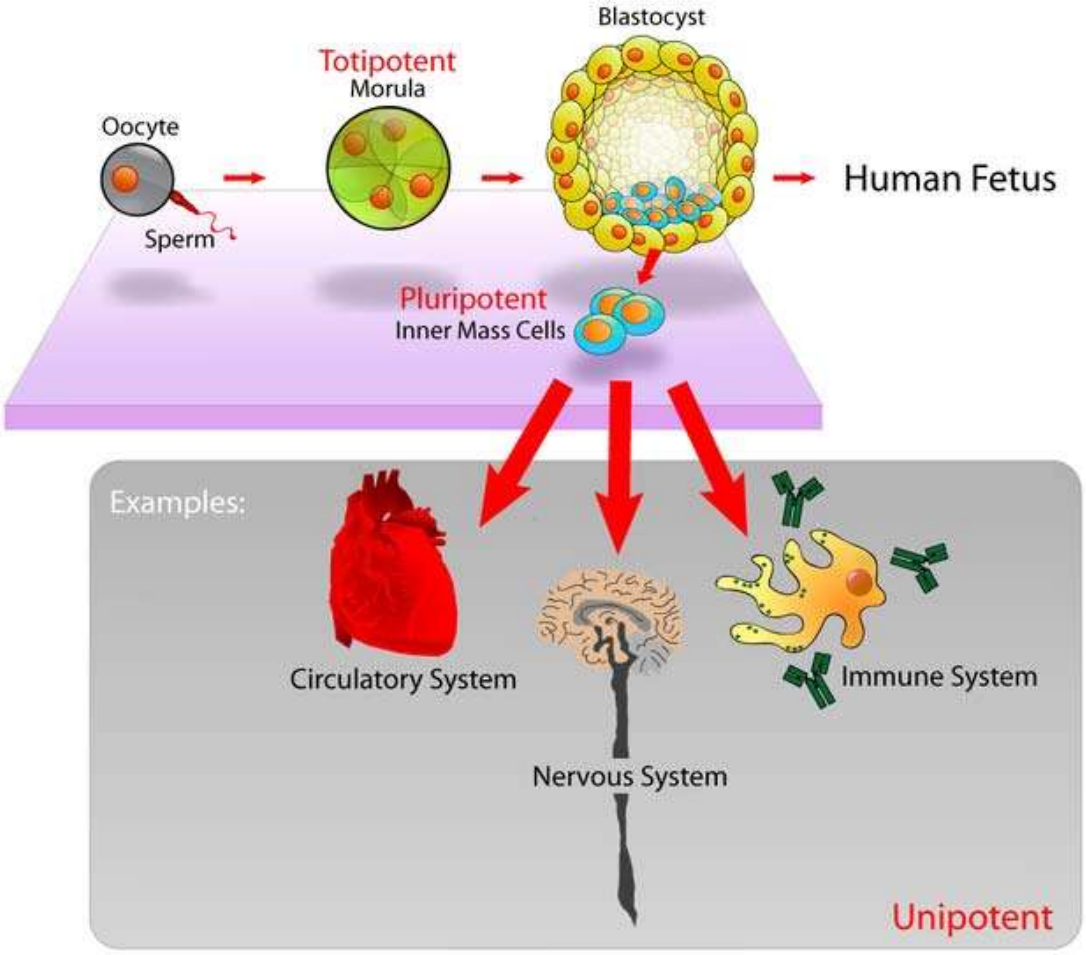
- *Self-renewal* - the ability to go through numerous cycles of cell division while maintaining the undifferentiated state.
- *Potency* - the capacity to differentiate into specialized cell types. In the strictest sense, this requires stem cells to be either totipotent or pluripotent - to be able to give rise to any mature cell type, although multipotent or unipotent progenitor cells are sometimes referred to as stem cells.

Self-renewal

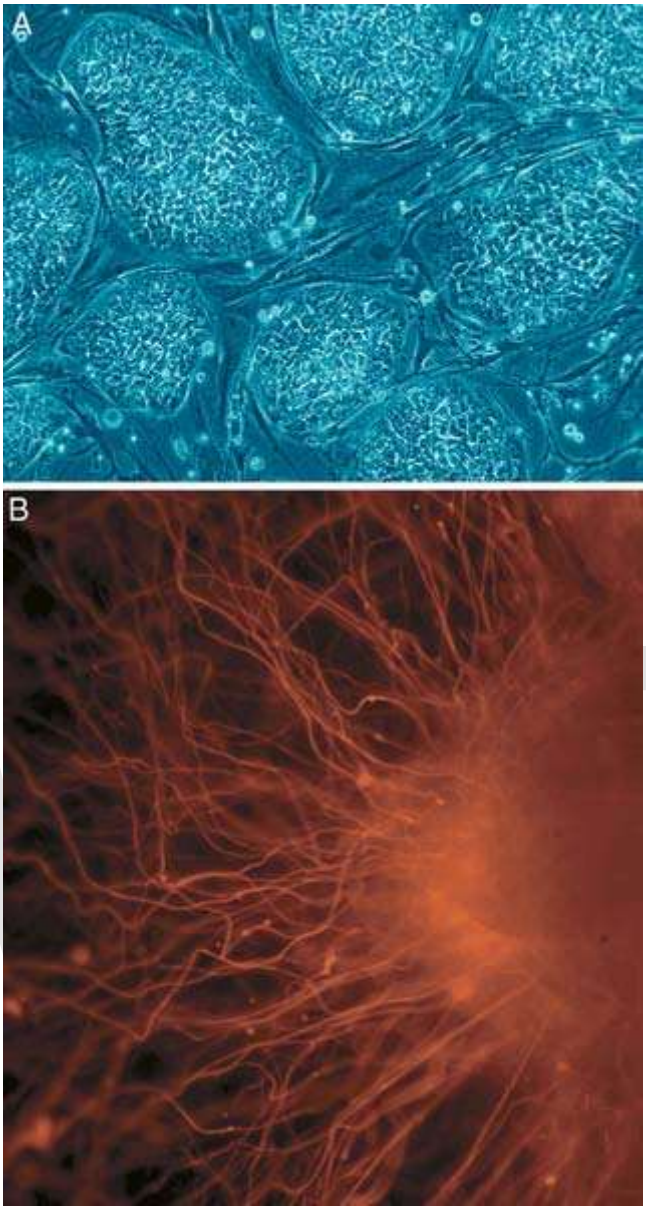
Two mechanisms exist to ensure that the stem cell population is maintained:

1. Obligatory asymmetric replication - a stem cell divides into one father cell that is identical to the original stem cell, and another daughter cell that is differentiated
2. Stochastic differentiation - when one stem cell develops into two differentiated daughter cells, another stem cell undergoes mitosis and produces two stem cells identical to the original.

Potency definitions



Pluripotent, embryonic stem cells originate as inner mass cells within a blastocyst. The stem cells can become any tissue in the body, excluding a placenta. Only the morula's cells are totipotent, able to become all tissues and a placenta.



Human embryonic stem cells
A: Cell colonies that are not yet differentiated.
B: Nerve cell

Potency specifies the differentiation potential (the potential to differentiate into different cell types) of the stem cell.

- Totipotent (a.k.a omnipotent) stem cells can differentiate into embryonic and extraembryonic cell types. Such cells can construct a complete, viable, organism. These cells are produced from the fusion of an egg and sperm cell. Cells produced by the first few divisions of the fertilized egg are also totipotent.
- Pluripotent stem cells are the descendants of totipotent cells and can differentiate into nearly all cells, i.e. cells derived from any of the three germ layers.

- Multipotent stem cells can differentiate into a number of cells, but only those of a closely related family of cells.
- Oligopotent stem cells can differentiate into only a few cells, such as lymphoid or myeloid stem cells.
- Unipotent cells can produce only one cell type, their own, but have the property of self-renewal which distinguishes them from non-stem cells (e.g. muscle stem cells).

Semen

Human semen in a Petri dish.

Semen is an organic fluid, also known as *seminal fluid*, that may contain spermatozoa. It is secreted by the gonads (sexual glands) and other sexual organs of male or hermaphroditic animals and can fertilize female ova. In humans, seminal fluid contains several components besides spermatozoa: proteolytic and other enzymes as well as fructose are elements of seminal fluid which promote the survival of spermatozoa and provide a medium through which they can move or "swim". The process that results in the discharge of semen is called *ejaculation*.

Physiological aspects

Internal and external fertilization

Depending on the species, spermatozoa can fertilize ova externally or internally. In external fertilization, the spermatozoa fertilize the ova directly, outside of the female's sexual organs. Female fish, for example, spawn ova into their aquatic environment, where they are fertilized by the semen of the male fish.

During internal fertilization, however, fertilization occurs inside the female's sexual organs. Internal fertilization takes place after insemination of a female by a male through copulation. In low vertebrates (amphibians, reptiles, birds and monotreme mammals), copulation is achieved through the physical mating of the cloaca of the male and female. In marsupial and placental mammals, copulation occurs through the vagina.

Composition of human semen

During the process of ejaculation, sperm passes through the ejaculatory ducts and mixes with fluids from the seminal vesicles, the prostate, and the bulbourethral glands to form the semen. The seminal vesicles produce a yellowish viscous fluid rich in fructose and other substances that makes up about 70% of human semen. The prostatic secretion, influenced by dihydrotestosterone, is a whitish (sometimes clear), thin fluid containing proteolytic enzymes, citric acid, acid phosphatase and lipids. The bulbourethral glands secrete a clear secretion into the lumen of the urethra to lubricate it.

Sertoli cells, which nurture and support developing spermatocytes, secrete a fluid into seminiferous tubules that helps transport sperm to the genital ducts. The ductuli efferentes possess cuboidal cells with microvilli and lysosomal granules that modify the semen by reabsorbing some fluid. Once the semen enters the ductus epididymis the principle cells, which contain pinocytotic vessels indicating fluid reabsorption, secrete glycerophosphocholine which most likely inhibits premature capacitation. The accessory genital ducts, the seminal vesicle, prostate glands, and the bulbourethral glands, produce most of the seminal fluid.

Seminal plasma of humans contains a complex range of organic and inorganic constituents.

The seminal plasma provides a nutritive and protective medium for the spermatozoa during their journey through the female reproductive tract. The normal environment of the vagina is a hostile one for sperm cells, as it is very acidic (from the native microflora producing lactic acid), viscous, and patrolled by immune cells. The components in the seminal plasma attempt to compensate for this hostile environment. Basic amines such as putrescine, spermine, spermidine and cadaverine are responsible for the smell and flavor of semen. These alkaline bases counteract the acidic environment of the vaginal canal, and protect DNA inside the sperm from acidic denaturation.

The components and contributions of semen are as follows:

Gland	Approximate %	Description
testes	2–5%	Approximately 200- to 500-million spermatozoa (also called *sperm* or *spermatozoans*), produced in the testes, are released per ejaculation.
seminal vesicle	65–75%	amino acids, citrate, enzymes, flavins, fructose (the main energy source of sperm cells, which rely entirely on sugars from the seminal plasma for energy), phosphorylcholine, prostaglandins (involved in suppressing an immune response by the female against the foreign semen), proteins, vitamin C
prostate	25–30%	acid phosphatase, citric acid, fibrinolysin, prostate specific antigen, proteolytic enzymes, zinc (the zinc level is about 135±40 micrograms/ml for healthy men. Zinc serves to help to stabilize the DNA-containing chromatin in the sperm cells. A zinc deficiency may result in lowered fertility because of increased sperm fragility. Zinc deficiency can also adversely affect spermatogenesis.)
bulbourethral glands	< 1%	galactose, mucus (serve to increase the mobility of sperm cells in the vagina and cervix by creating a less viscous channel for the sperm cells to swim through, and preventing their diffusion out of the semen. Contributes to the cohesive jelly-like texture of semen.), pre-ejaculate, sialic acid

A 1992 World Health Organization report described normal human semen as having a volume of 2 ml or greater, pH of 7.2 to 8.0, sperm concentration of 20×10^6 spermatozoa/ml or more, sperm count of 40×10^6 spermatozoa per ejaculate or more, and motility of 50% or more with forward progression (categories a and b) of 25% or more with rapid progression (category a) within 60 minutes of ejaculation.

Appearance and consistency of human semen

Semen is typically translucent with white, grey or even yellowish tint. Blood in the semen can cause a pink or reddish colour, known as *hematospermia*, and may indicate a medical problem which should be evaluated by a doctor if the symptom persists.

After ejaculation, the latter part of the ejaculated semen coagulates immediately, forming globules, while the earlier part of the ejaculate typically does not. After a period typically ranging from 15 – 30 minutes, Prostate-specific antigen present in the semen causes the decoagulation of the seminal coagulum. It is postulated that the initial clotting helps keep the semen in the vagina, while liquefaction frees the sperm to make their journey to the ova.

Semen quality

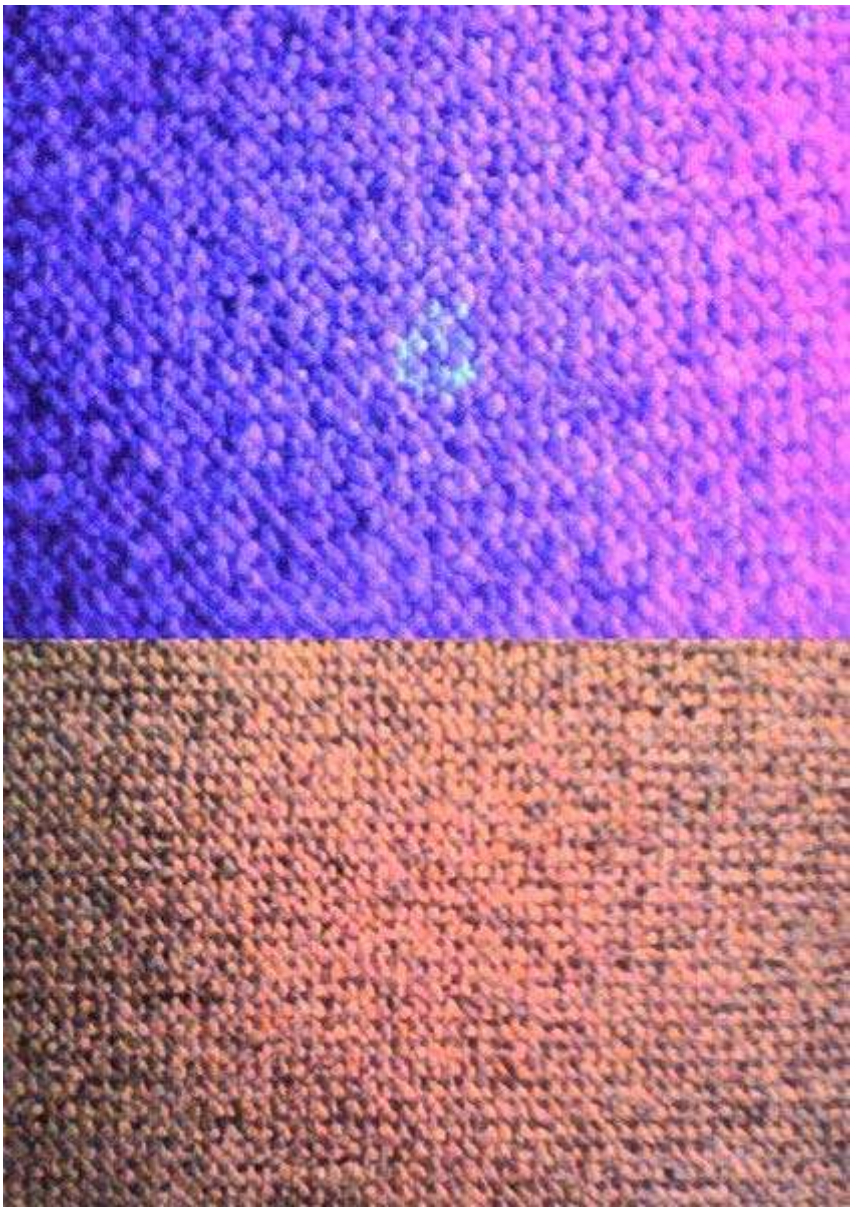
Semen quality is a measure of the ability of semen to accomplish fertilization. Thus, it is a measure of fertility in a man. It is the sperm in the semen that is the fertile component, and therefore semen quality involves both sperm quantity and sperm quality.

Blood in the semen (hematospermia)

The presence of blood in semen or hematospermia may be undetectable (it only can be seen microscopically) or visible in the fluid. Its cause could be the result of inflammation, infection, blockage, or injury of the male reproductive tract or a problem within the urethra, testicles, epididymis or prostate.

It usually clears up without treatment, or with antibiotics, but if persistent further semen analysis and other urogenital system tests might be needed to find out the cause.

Semen in espionage



Semen stain on carpet seen with and without ultraviolet light

When the British Secret Intelligence Service discovered that semen made a good invisible ink, Sir George Mansfield Smith-Cumming noted of his agents that "Every man (is) his own stylo".

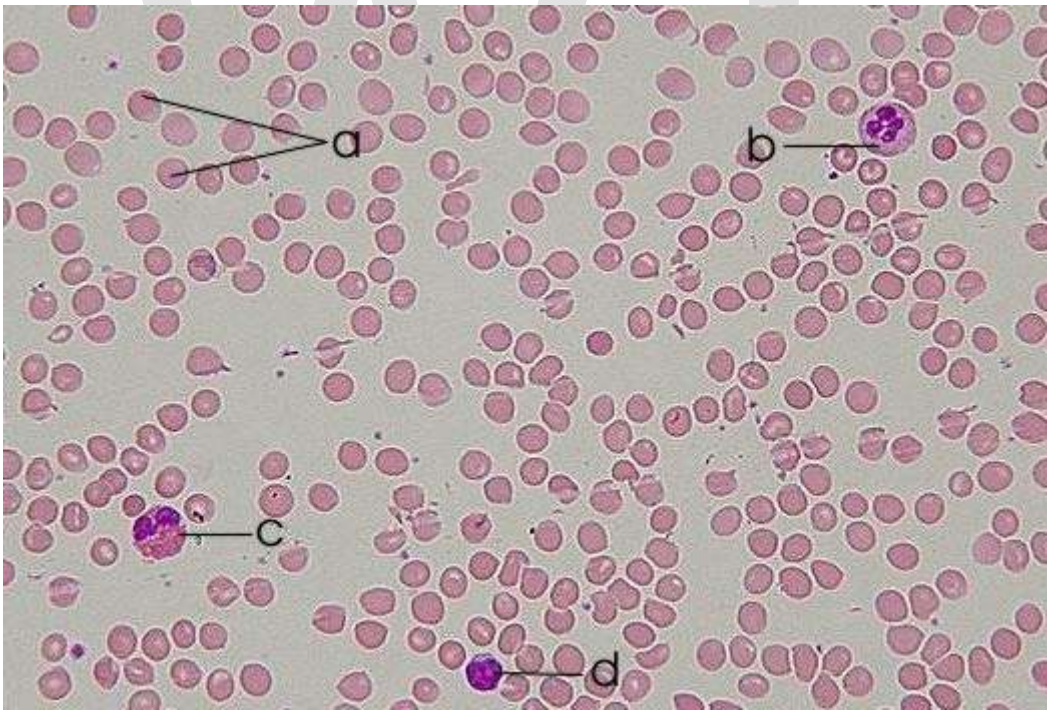
Semen ingestion

Some reasons for human ingestion of human or other semen are erotic gratification and physical and spiritual benefits. The most common way that swallowing of semen occurs is when fellatio or irrumatio are performed to climax.

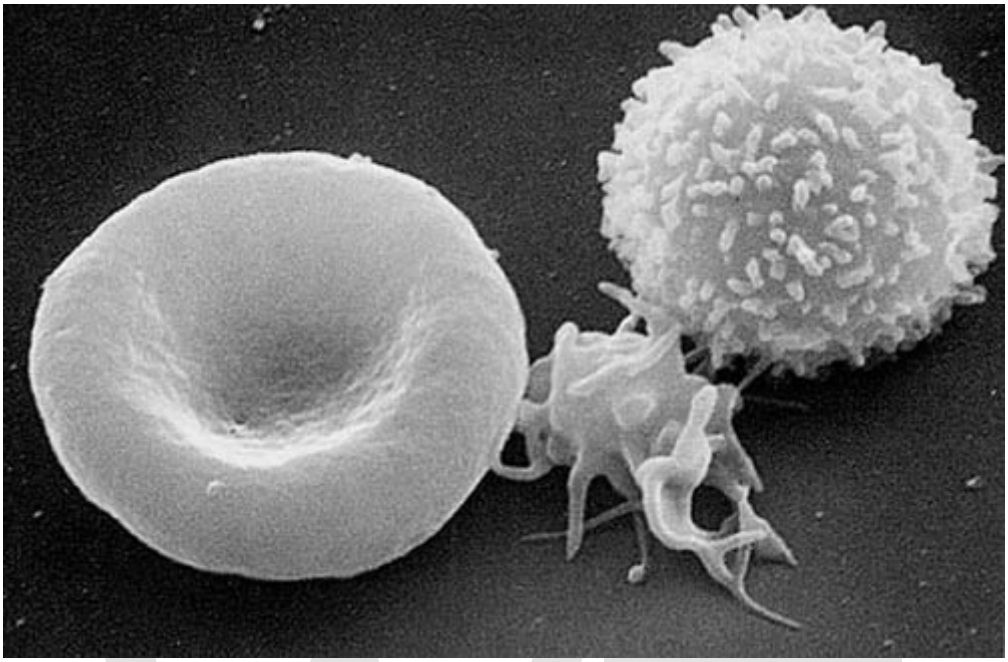
Nutritional value

Semen is primarily water, but contains trace amounts of almost every nutrient the human body uses. It has somewhat higher amounts of commonly deficient minerals, such as potassium, magnesium, and selenium. One typical ejaculation contains 150 mg of protein, 11 mg of carbohydrates, 6 mg fat, 3 mg cholesterol, 7% US RDA potassium and 3% US RDA copper and zinc. When metabolized, protein yields 4 kcal/g, carbohydrate also yields 4 kcal/g, and fat yields 9 kcal/g. Hence the food energy in the typical ejaculation is 0.7 kcal (2.9 kJ).

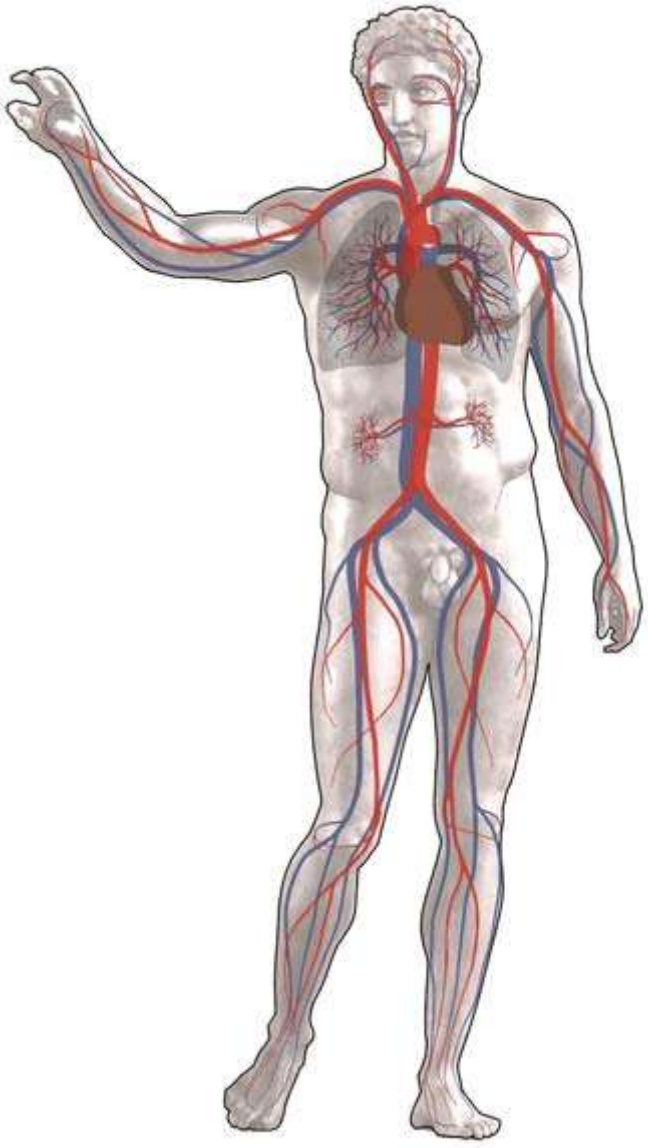
Blood



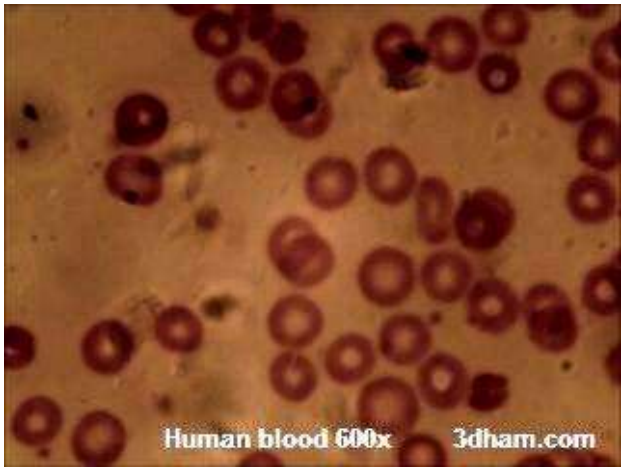
Human blood smear:
a – erythrocytes; b – neutrophil;
c – eosinophil; d – lymphocyte.



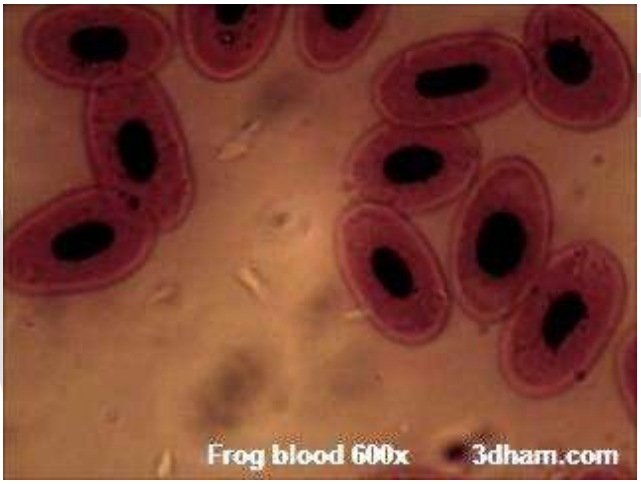
A scanning electron microscope (SEM) image of a normal red blood cell, a platelet, and a white blood cell.



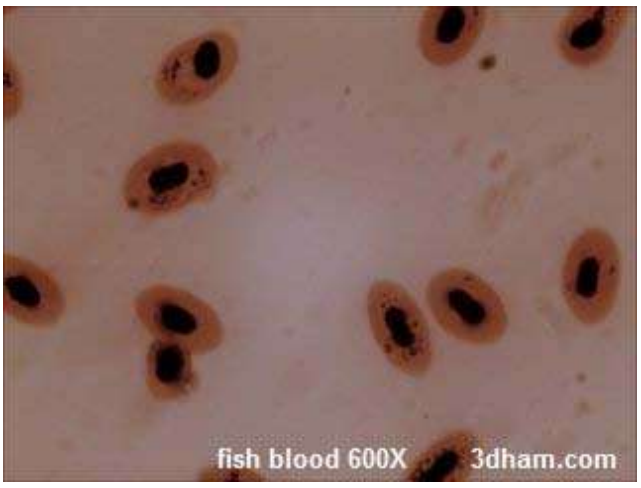
Blood circulation:
Red = oxygenated
Blue = deoxygenated



Human blood magnified 600 times



Frog blood magnified 600 times



Fish blood magnified 600 times

Blood is a specialized bodily fluid that delivers necessary substances to the body's cells (in animals) – such as nutrients and oxygen – and transports waste products away from those same cells.

In vertebrates, it is composed of blood cells suspended in a liquid called blood plasma. Plasma, which constitutes 55% of blood fluid, is mostly water (92% by volume), and contains dissipated proteins, glucose, mineral ions, hormones, carbon dioxide (plasma being the main medium for excretory product transportation), platelets and blood cells themselves. The blood cells present in blood are mainly red blood cells (also called RBCs or erythrocytes) and white blood cells, including leukocytes and platelets. The most abundant cells in vertebrate blood are red blood cells. These contain hemoglobin, an iron-containing protein, which facilitates transportation of oxygen by reversibly binding to this respiratory gas and greatly increasing its solubility in blood. In contrast, carbon dioxide is almost entirely transported extracellularly dissolved in plasma as bicarbonate ion.

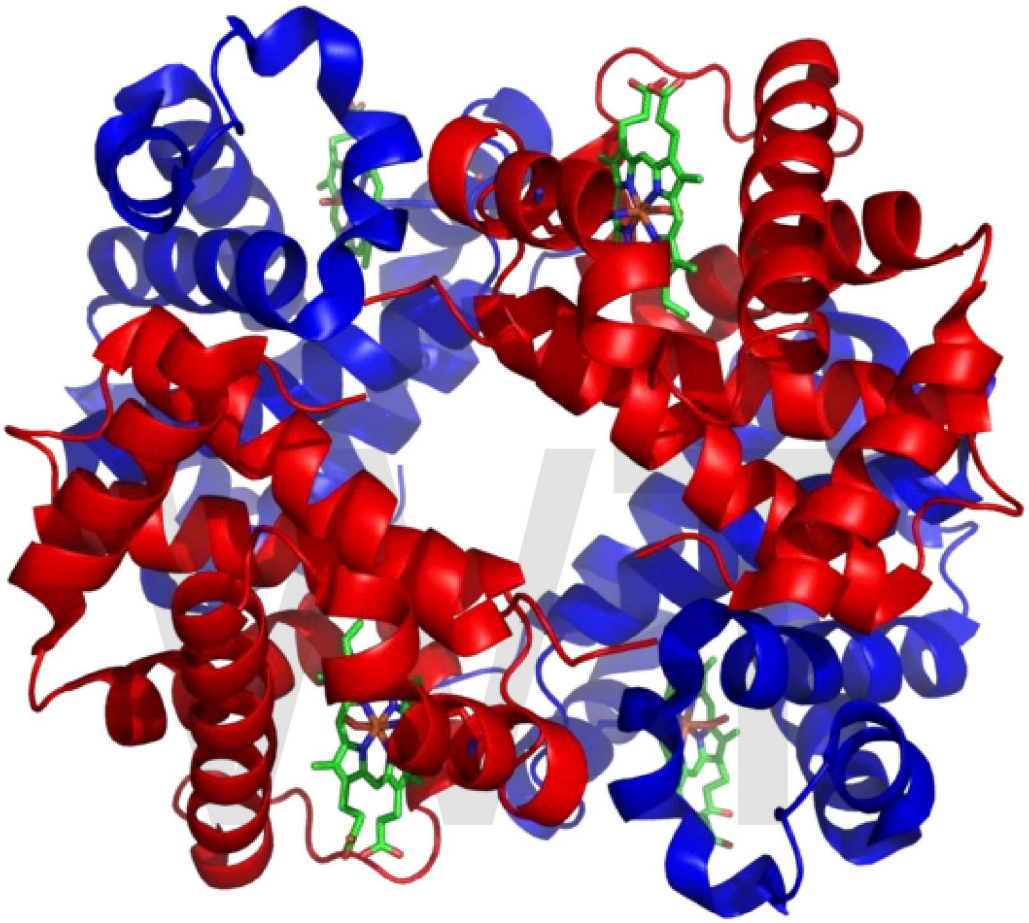
Vertebrate blood is bright red when its hemoglobin is oxygenated. Some animals, such as crustaceans and mollusks, use hemocyanin to carry oxygen, instead of hemoglobin. Insects and some molluscs use a fluid called hemolymph instead of blood, the difference being that hemolymph is not contained in a closed circulatory system. In most insects, this "blood" does not contain oxygen-carrying molecules such as hemoglobin because their bodies are small enough for their tracheal system to suffice for supplying oxygen.

Jawed vertebrates have an adaptive immune system, based largely on white blood cells. White blood cells help to resist infections and parasites. Platelets are important in the clotting of blood. Arthropods, using hemolymph, have hemocytes as part of their immune system.

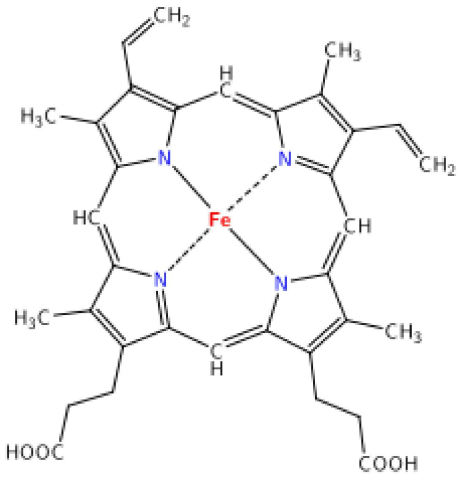
Blood is circulated around the body through blood vessels by the pumping action of the heart. In animals with lungs, arterial blood carries oxygen from inhaled air to the tissues of the body, and venous blood carries carbon dioxide, a waste product of metabolism produced by cells, from the tissues to the lungs to be exhaled.

Medical terms related to blood often begin with ***hemo-*** or ***hemato-*** (also spelled ***haemo-*** and ***haemato-***) from the Ancient Greek word αἷμα (*haima*) for "blood". In terms of anatomy and histology, blood is considered a specialized form of connective tissue, given its origin in the bones and the presence of potential molecular fibers in the form of fibrinogen.

Functions



Hemoglobin
green = heme groups
red & blue = protein subunits



Heme

Blood performs many important functions within the body including:

- Supply of oxygen to tissues (bound to hemoglobin, which is carried in red cells)
- Supply of nutrients such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins (e.g., blood lipids))
- Removal of waste such as carbon dioxide, urea, and lactic acid
- Immunological functions, including circulation of white blood cells, and detection of foreign material by antibodies
- Coagulation, which is one part of the body's self-repair mechanism (blood clotting after an open wound in order to stop bleeding)
- Messenger functions, including the transport of hormones and the signaling of tissue damage
- Regulation of body pH
- Regulation of core body temperature
- Hydraulic functions

Constituents of human blood

Two tubes of EDTA-anticoagulated blood.
Left tube: after standing, the RBCs have settled at the bottom of the tube.
Right tube: contains freshly drawn blood.

Blood accounts for 8% of the human body weight, with an average density of approximately 1060 kg/m^3, very close to pure water's density of 1000 kg/m^3. The average adult has a blood volume of roughly 5 liters (1.3 gal), composed of plasma and several kinds of cells (occasionally called *corpuscles*); these formed elements of the blood are erythrocytes (red blood cells), leukocytes (white blood cells), and thrombocytes (platelets). By volume, the red blood cells constitute about 45% of whole blood, the plasma about 54.3%, and white cells about 0.7%.

Whole blood (plasma and cells) exhibits non-Newtonian, viscoelastic fluid dynamics; its flow properties are adapted to flow effectively through tiny capillary blood vessels with less resistance than plasma by itself. In addition, if all human hemoglobin were free in the plasma rather than being contained in RBCs, the circulatory fluid would be too viscous for the cardiovascular system to function effectively.

Cells

One microliter of blood contains:

- **4.7 to 6.1 million (male), 4.2 to 5.4 million (female) erythrocytes:** In most mammals, mature red blood cells lack a nucleus and organelles. They contain the blood's hemoglobin and distribute oxygen. The red blood cells (together with endothelial vessel cells and other cells) are also marked by glycoproteins that define the different blood types. The proportion of blood occupied by red blood cells is referred to as the hematocrit, and is normally about 45%. The combined surface area of all red blood cells of the human body would be roughly 2,000 times as great as the body's exterior surface.
- **4,000–11,000 leukocytes:** White blood cells are part of the immune system; they destroy and remove old or aberrant cells and cellular debris, as well as attack infectious agents (pathogens) and foreign substances. The cancer of leukocytes is called leukemia.
- **200,000–500,000 thrombocytes:** thrombocytes, also called platelets, are responsible for blood clotting (coagulation). They change fibrinogen into fibrin. This fibrin creates a mesh onto which red blood cells collect and clot, which then stops more blood from leaving the body and also helps to prevent bacteria from entering the body.

Constitution of normal blood

Parameter	Value
Hematocrit	45 ± 7 (38–52%) for males 42 ± 5 (37–47%) for females
pH	7.35–7.45
base excess	−3 to +3
PO_2	10–13 kPa (80–100 mm Hg)
PCO_2	4.8–5.8 kPa (35–45 mm Hg)

HCO_3^-	21–27 mM
Oxygen saturation	Oxygenated: 98–99% Deoxygenated: 75%

Plasma

About 55% of whole blood is blood plasma, a fluid that is the blood's liquid medium, which by itself is straw-yellow in color. The blood plasma volume totals of 2.7–3.0 liters (2.8–3.2 quarts) in an average human. It is essentially an aqueous solution containing 92% water, 8% blood plasma proteins, and trace amounts of other materials. Plasma circulates dissolved nutrients, such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins), and removes waste products, such as carbon dioxide, urea, and lactic acid.

Other important components include:

- Serum albumin
- Blood-clotting factors (to facilitate coagulation)
- Immunoglobulins (antibodies)
- lipoprotein particles
- Various other proteins
- Various electrolytes (mainly sodium and chloride)

The term **serum** refers to plasma from which the clotting proteins have been removed. Most of the proteins remaining are albumin and immunoglobulins.

Narrow range of pH values

Blood pH is regulated to stay within the narrow range of 7.35 to 7.45, making it slightly alkaline. Blood that has a pH below 7.35 is too acidic, whereas blood pH above 7.45 is too alkaline. Blood pH, partial pressure of oxygen (pO_2), partial pressure of carbon dioxide (pCO_2), and HCO_3^- are carefully regulated by a number of homeostatic mechanisms, which exert their influence principally through the respiratory system and the urinary system in order to control the acid-base balance and respiration. An arterial blood gas will measure these. Plasma also circulates hormones transmitting their messages to various tissues. The list of normal reference ranges for various blood electrolytes is extensive.

Bones are especially affected by blood pH as they tend to be used as a mineral source for pH buffering. Consuming a high ratio of animal protein to vegetable protein is implicated in bone loss in women.

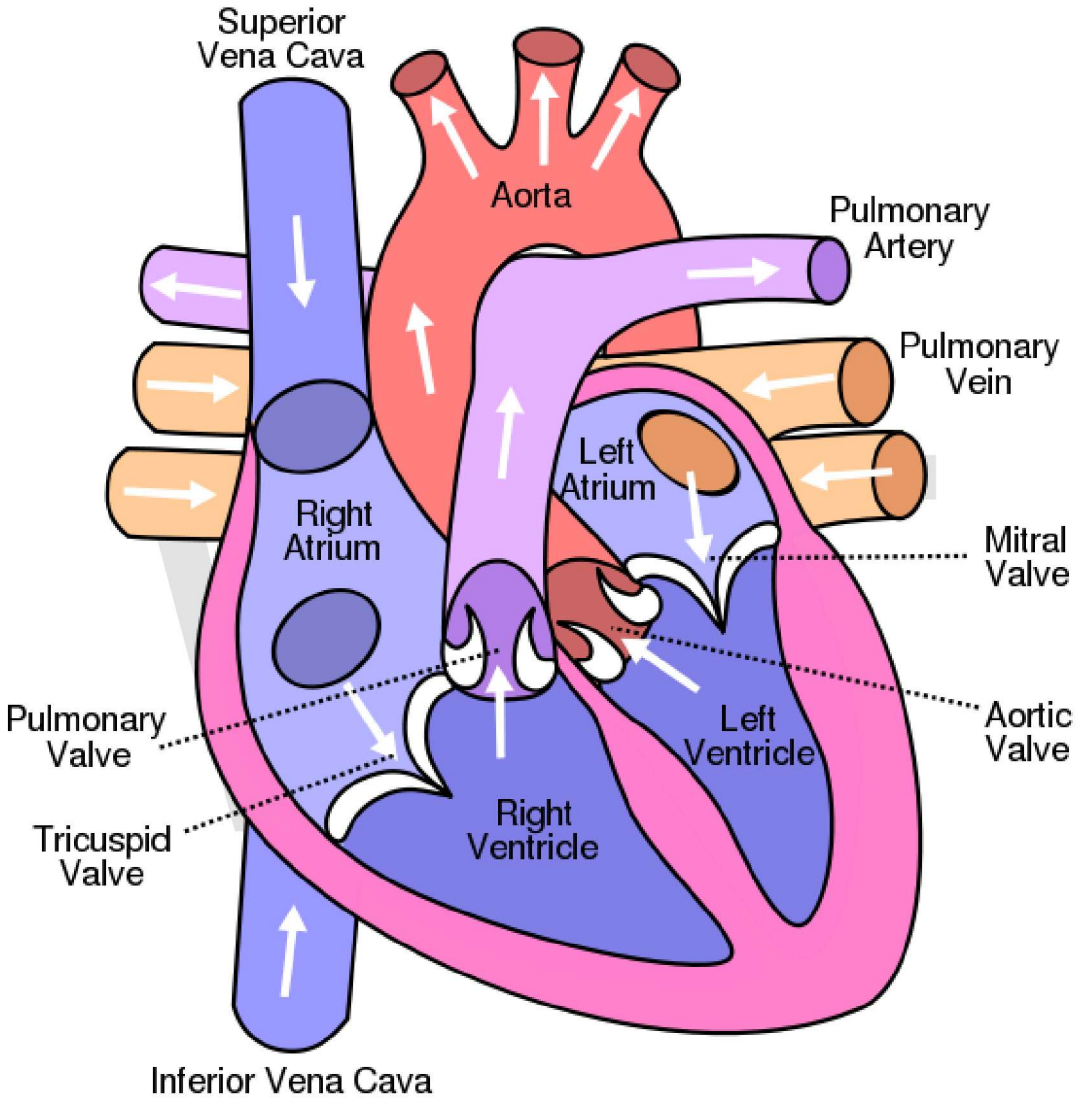
Blood in non-human vertebrates

Human blood is typical of that of mammals, although the precise details concerning cell numbers, size, protein structure, and so on, vary somewhat between species. In non-mammalian vertebrates, however, there are some key differences:

- Red blood cells of non-mammalian vertebrates are flattened and ovoid in form, and retain their cell nuclei
- There is considerable variation in the types and proportions of white blood cells; for example, acidophils are generally more common than in humans
- Platelets are unique to mammals; in other vertebrates, small, nucleated, spindle cells are responsible for blood clotting instead

Physiology

Cardiovascular system



The circulation of blood through the human heart

Blood is circulated around the body through blood vessels by the pumping action of the heart. In humans, blood is pumped from the strong left ventricle of the heart through arteries to peripheral tissues and returns to the right atrium of the heart through veins. It then enters the right ventricle and is pumped through the pulmonary artery to the lungs and returns to the left atrium through the pulmonary veins. Blood then enters the left ventricle to be circulated again. Arterial blood carries oxygen from inhaled air to all of the cells of the body, and venous blood carries carbon dioxide, a waste product of metabolism by cells, to the lungs to be exhaled. However, one exception includes

pulmonary arteries, which contain the most deoxygenated blood in the body, while the pulmonary veins contain oxygenated blood.

Additional return flow may be generated by the movement of skeletal muscles, which can compress veins and push blood through the valves in veins toward the right atrium.

The blood circulation was famously described by William Harvey in 1628.

Production and degradation of blood cells

In vertebrates, the various cells of blood are made in the bone marrow in a process called hematopoiesis, which includes erythropoiesis, the production of red blood cells; and myelopoiesis, the production of white blood cells and platelets. During childhood, almost every human bone produces red blood cells; as adults, red blood cell production is limited to the larger bones: the bodies of the vertebrae, the breastbone (sternum), the ribcage, the pelvic bones, and the bones of the upper arms and legs. In addition, during childhood, the thymus gland, found in the mediastinum, is an important source of lymphocytes. The proteinaceous component of blood (including clotting proteins) is produced predominantly by the liver, while hormones are produced by the endocrine glands and the watery fraction is regulated by the hypothalamus and maintained by the kidney.

Healthy erythrocytes have a plasma life of about 120 days before they are degraded by the spleen, and the Kupffer cells in the liver. The liver also clears some proteins, lipids, and amino acids. The kidney actively secretes waste products into the urine.

Chapter- 4

Semen Cryopreservation, Oocyte Cryopreservation and Ovarian Tissue Cryopreservation

Semen cryopreservation

Semen cryopreservation is a procedure to preserve sperm cells. Semen can be used successfully indefinitely after cryopreservation. For human sperm, the longest reported successful storage is 21 years. It can be used for sperm donation where the recipient wants the treatment in a different time or place, or for men undergoing a vasectomy to still have the option to have children.

Freezing

The most common cryoprotectant used for semen is glycerol (10% in culture medium). Often sucrose or other di-, trisaccharides are added to glycerol solution. Cryoprotectant media may be supplemented with either egg yolk or soy lecithin, with the two having no statistically significant differences compared to each other regarding motility, morphology, ability to bind to hyaluronate in vitro, or DNA integrity after thawing.

Semen is frozen using either a controlled-rate, slow-cooling method (slow programmable freezing or SPF) or a newer flash-freezing process known as vitrification. Vitrification gives superior post-thaw motility and cryosurvival than *slow programmable freezing*.

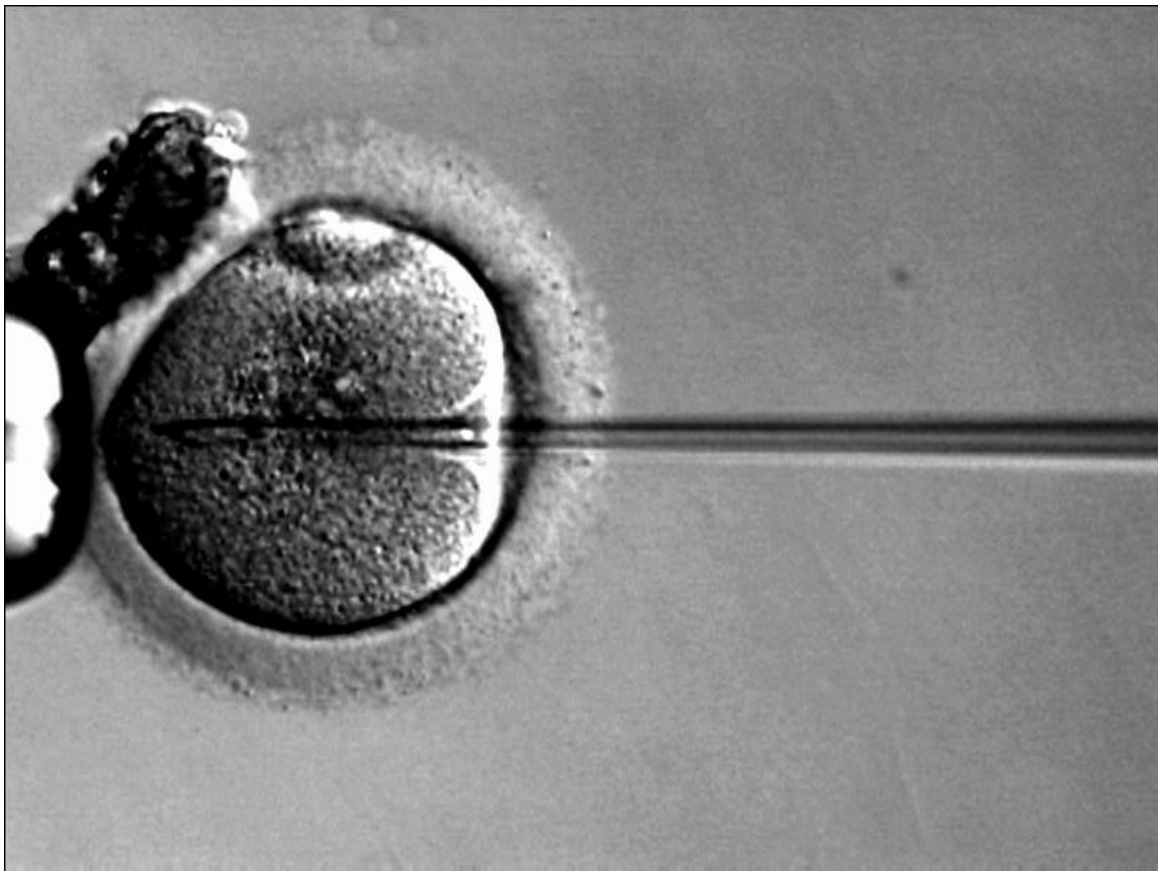
Thawing

Thawing at 40°C seems to result in optimal sperm motility. On the other hand, the exact thawing temperature seems to have only minor effect on sperm viability, acrosomal status, ATP content, and DNA.

Refreezing

In terms of the level of sperm DNA fragmentation, up to three cycles of freezing and thawing can be performed without causing a level of risk significantly higher than following a single cycle of freezing and thawing. This is provided that samples are refrozen in their original cryoprotectant and are not going through sperm washing or other alteration in between, and provided that they are separated by density gradient centrifugation or swim-up before use in assisted reproduction technology.

Oocyte cryopreservation



ICSI sperm injection into oocyte

Human **oocyte cryopreservation** (**egg freezing**) is a novel technology in which a woman's eggs (oocytes) are extracted, frozen and stored. Later, when she is ready to become pregnant, the eggs can be thawed, fertilized, and transferred to the uterus as embryos.

History

Cryopreservation itself has always played a central role in assisted reproductive technology. With the first cryopreservation of sperm in 1953 and of embryos thirty years later, these techniques have become routine. Dr Christopher Chen of Australia reported the world's first pregnancy in 1986 using previously frozen oocytes. This report stood alone for several years followed by studies reporting success rates using frozen eggs to be much lower than those of traditional in vitro fertilization (IVF) techniques using fresh oocytes. Then recently, two articles published in the journal, *Fertility and Sterility*, reported pregnancy rates using frozen oocytes that were comparable to those of cryopreserved embryos and even fresh embryos. These newer reports affirm that oocyte cryopreservation technology is advancing.

Indications

Oocyte cryopreservation is aimed at three particular groups of women: those diagnosed with cancer who have not yet begun chemotherapy or radiotherapy; those undergoing treatment with assisted reproductive technologies who do not consider embryo freezing an option; and those who would like to preserve their future ability to have children, either because they do not yet have a partner, or for other personal or medical reasons.

Over 50,000 reproductive-age women are diagnosed with cancer each year in the United States. Chemotherapy and radiotherapy are toxic for oocytes, leaving few, if any, viable eggs. Egg freezing offers women with cancer the chance to preserve their eggs so that they can have children in the future.

Oocyte cryopreservation is an important option for individuals undergoing IVF who object, either for religious or ethical reasons, to the practice of freezing embryos. Having the option to fertilize only as many eggs as will be utilized in the IVF process, and then freeze any remaining unfertilized eggs can be a positive solution. In this way, there are no excess embryos created, and there need be no disposition of unused frozen embryos, a practice which can create complex choices for certain individuals.

Egg freezing can also be beneficial for women who, for the purpose of education, career or other reasons, desire to postpone childbearing. Freezing eggs at an early age may ensure a chance for a future pregnancy.

Additionally, women with a family history of early menopause have an interest in fertility preservation. With egg freezing, they will have a frozen store of eggs, in the likelihood that their eggs are depleted at an early age.

Method

The egg retrieval process for oocyte cryopreservation is the same as that for in vitro fertilization. This includes one to several weeks of hormone injections that stimulate

ovaries to ripen multiple eggs. When the eggs are mature, a medication to trigger ovulation is given and the eggs are removed from the body using an ultrasound-guided needle through the vagina. The procedure is usually conducted under sedation. The eggs are immediately frozen.

The egg is the largest cell in the human body and contains a great amount of water. When the egg is frozen, the ice crystals that form can destroy the integrity of the cell. To prevent this, the egg must be dehydrated prior to freezing. This is done using cryoprotectants which replace the water within the cell and inhibit the formation of ice crystals.

Eggs (oocytes) are frozen using either a controlled-rate, slow-cooling method or a newer flash-freezing process known as vitrification. The slow-cooling method is the most practiced of embryo freezing techniques. Vitrification is much faster but requires higher concentrations of cryoprotectants to be added. The result of vitrification is a solid glass-like cell, free of ice crystals. There are differing schools of thought on which freezing method is theoretically superior for oocytes but large amounts of comparative data on the two methods is lacking at this time. With regard to slow freezing of embryos, a study involving 23 countries showed almost 42,000 'slow frozen' (as opposed to 'vitrified') human embryo transfers were performed during 2001 in Europe (Andersen et al. 2005). In addition, it is estimated that between 300,000 and 500,000 successful human births have resulted worldwide from the transfer of previously ‘slow frozen’ embryos performed from the mid-1970s to 2006.

Once frozen, the zona pellucida, or shell of the egg hardens. Thus, currently, when eggs are thawed, a special fertilization procedure is performed by an embryologist whereby sperm is injected directly into the egg with a needle rather than allowing sperm to penetrate naturally by placing it around the egg in a dish. This injection technique is called ICSI (Intracytoplasmic Sperm Injection) and is also used in IVF.

Success rates

The percentage of transferred cycles is somewhat lower in frozen cycles compared with fresh cycles (approx. 80% and 90%, respectively).

Two recent studies showed that the rate of birth defects and chromosomal defects when using cryopreserved oocytes is consistent with that of natural conception.

Recent modifications in protocol regarding cryoprotectant composition, temperature and storage methods have had a large impact on the technology, and while it is still considered an experimental procedure, it is quicly becoming an option for women. Slow freezing traditionally has been the most commonly used method to cryopreserve oocytes, and is the method that has resulted in the most babies born from frozen oocytes worldwide. Ultra-rapid freezing or vitrification represents a potential alternative freezing method.

In the fall of 2009, The American Society for Reproductive Medicine (ASRM) issued an opinion on oocyte cryopreservation concluding that the science holds "great promise for applications in oocyte donation and fertility preservation" because recent laboratory modifications have resulted in improved oocyte survival, fertilization, and pregnancy rates from frozen-thawed oocytes in IVF. The ASRM noted that from the limited research performed to date, there does not appear to be an increase in chromosomal abnormalities, birth defects, or developmental deficits in the children born from cryopreserved oocytes. The ASRM recommends that, pending further research, oocyte cryopreservation should be introduced into clinical practice on an investigational basis and under the guidance of an Institutional Review Board (IRB). As with any new technology, safety and efficacy must be evaluated and demonstrated through continued research.

Cost

The cost of egg freezing, (including the embryo transfer) is comparable to that of IVF and ranges from $12,000 to $20,000. Egg storage can be several hundred dollars or more per year.

Ovarian tissue cryopreservation

Ovarian tissue cryopreservation is cryopreservation of tissue of the ovary of a female.

Indications

Cryopreservation of ovarian tissue is of interest to women who want fertility preservation beyond the natural limit, or whose reproductive potential is threatened by cancer therapy, for example in hematologic malignancies or breast cancer. It can be performed on prepubertal girls at risk for premature ovarian failure, and this procedure is as feasible and safe as comparable operative procedures in children.

Procedure

The procedure is to take a part of the ovary and carry out slow freezing before storing it in liquid nitrogen whilst therapy is undertaken. Tissue can then be thawed and implanted near the fallopian, either orthotopic (on the natural location) or heterotopic (on the abdominal wall), where it starts to produce new eggs, allowing normal conception to take place. A study of 60 procedures concluded that ovarian tissue harvesting appears to be safe. The ovarian tissue may also be transplanted into mice that are immunocompromised (SCID mice) to avoid graft rejection, and tissue can be harvested later when mature follicles have developed.

Strips of cortical ovarian tissue can also be cryopreserved, but it must be re-implanted into the body to allow the encapsulated immature follicles to complete their maturation. Furthermore, ovarian tissue is fragile under hard freezing conditions and putting it back into the body carries the risk of re-introducing cancerous cells.

History

First ovarian transplant with cryopreserved ovarian tissue was performed by Dr Kutluk Oktay in 1999. In Sep 2004 *Prof Donnez* of Louvain in Belgium reported the first successful ovarian birth from slow, or controlled rate, frozen ovarian tissue. In 1997 samples of ovarian cortex were taken from a woman with Hodgkin's lymphoma and cryo-preserved in a rate freezer (Planer, UK)and stored in liquid Nitrogen. Chemotherapy was initiated and after the patient had premature ovarian failure. In 2003, after freeze-thawing, orthotopic autotransplantation of ovarian cortical tissue was done by laparoscopy and five months after reimplantation signs indicated recovery of regular ovulatory cycles. Eleven months after re-implantation a viable intrauterine pregnancy was confirmed, which resulted in a live birth – *Tamara*.

Chapter- 5

Cryobiology

Cryobiology is the branch of biology that studies the effects of low temperatures on living things. The word cryobiology is derived from the Greek words "cryo" = cold, "bios" = life, and "logos" = science. In practice, cryobiology is the study of biological material or systems at temperatures below normal. Materials or systems studied may include proteins, cells, tissues, organs, or whole organisms. Temperatures may range from moderately hypothermic conditions to cryogenic temperatures.

Definitions/Distinctions

Cryobiology
: is the study of life at low temperatures.

Cryogenics
: is the branch of physics and engineering that studies the production and use of very low temperatures. Cryogenics is not cryonics, although people often confuse them.

Cryonics
: is the low temperature preservation of humans and mammals with the intention of future revival. Cryonics is not part of mainstream cryobiology. Cryonics still depends heavily on speculative future technology which may or may not be invented.

Cryopreservation
: is a technology whereby cells, whole tissues, or embryos are preserved by cooling to temperatures below the freezing point of water.

Major areas of study in cryobiology

6 major areas of study in cryobiology can be identified:

1. Study of cold-adaptation of microorganisms, plants (= cold hardiness), and animals, both invertebrates and vertebrates (= hibernation).
2. Cryopreservation of cells, tissues, gametes, and embryos of animal and human origin for (medical) purposes of long-term storage. This usually requires the addition of substances which protect the cells during freezing and thawing (cryoprotectants).

3. Preservation of organs under hypothermic conditions for transplantation.
4. Lyophilization (freeze-drying) of pharmaceuticals.
5. Cryosurgery, a (minimally) invasive approach for the destruction of unhealthy tissue using cryogenic gases/fluids.
6. Physics of supercooling, ice nucleation/growth and mechanical engineering aspects of heat transfer during cooling and warming.

Cryopreservation in nature

Many living organisms are able to tolerate prolonged periods of time at temperatures below the freezing point of water. Most living organisms accumulate cryoprotectants such as anti-nucleating proteins, polyols, and glucose to protect themselves against frost damage by sharp ice crystals. Most plants, in particular, can safely reach temperatures of −4 °C to −12 °C.

Bacteria

Three species of bacteria, Carnobacterium pleistocenium, as well as Chryseobacterium greenlandensis and Herminiimonas glaciei, have reportedly been revived after surviving for thousands of years frozen in ice. Certain bacteria, notably *Pseudomonas syringae*, produce specialized proteins that serve as potent ice nucleators, which they use to force ice formation on the surface of various fruits and plants at about −2 °C. The freezing causes injuries in the epithelia and makes the nutrients in the underlying plant tissues available to the bacteria.

Plants

Many plants undergo a process called hardening which allows them to survive temperatures below 0 degrees C for weeks to months.

Animals

Invertebrates

Nematodes that survive below 0 degrees C include Trichostrongylus colubriformis and Panagrolaimus davidi. Cockroach nymphs (Periplaneta japonica) survive short periods of freezing at -6 to -8 degrees C. The red flat bark beetle (Cucujus clavipes) can survive after being frozen to -150 C. The fungus gnat Exechia nugatoria can survive after being frozen to -50 C, by a unique mechanism whereby ice crystals form in the body but not the head. Another freeze-tolerant beetle is Upis ceramboides. Another invertebrate that is tolerant to temperatures down to -273 C is the water bear, an extremophile.

The larvae of Haemonchus contortus, a nematode, can survive 44 weeks frozen at -196 C.

Vertebrates

For the wood frog (Rana sylvatica), in the winter, as much as 45% of its body may freeze and turn to ice. "Ice crystals form beneath the skin and become interspersed among the body's skeletal muscles. During the freeze the frog's breathing, blood flow, and heart beat cease. Freezing is made possible by specialized proteins and glucose, which prevent intracellular freezing and dehydration." The wood frog can survive up to 11 days frozen at -4 C.

Other vertebrates that survive at body temperatures below 0 C include painted turtles (Chrysemys picta), Gray tree frog (Hyla versicolor), Box turtles (Terrapene carolina)- 48 hours at -2 C, Spring peeper (Pseudacris crucifer), Garter snakes (Thamnophis sirtalis)- 24 hours at -1.5 C, the chorus frog (Pseudacris triseriata), Siberian salamander (Salamandrella keyserlingii), 24 hours at -15.3 C , Antarctic fish such as Pagothenia borchgrevinki and the European common lizard (Lacerta vivipara).

Professor Joshua Barr of the Staines Cyrobiology Laboratory in Middlesex (UK) has been carrying out experiments with British tree frogs to discover whether they also exhibit tolerance to very low temperatures in the same way as exhibited by American Tree Frogs. Thus far results have been inconclusive, however Professor Barr is optimistic about the future.

Hibernating Arctic ground squirrels may have abdominal temperatures as low as −2.9 °C (27 °F), maintaining sub-zero abdominal temperatures for more than three weeks at a time, although the temperatures at the head and neck remain at 0 C or above.

Applied cryobiology

Historical background

Boyle

Cryobiology history can be traced back to antiquity. As early as in 2500 BC low temperatures were used in Egypt in medicine. The use of cold was recommended by Hippocrates to stop bleeding and swelling. With the emergence of modern science, Robert Boyle studied the effects of low temperatures on animals.

In 1949 bull sperm was cryopreserved for the first time by a team of scientists led by Christopher Polge (1926–2006). This led to a much wider use of cryopreservation today, with many organs, tissues and cells routinely stored at low temperatures. Large organs such as hearts are usually stored and transported, for short times only, at cool but not freezing temperatures for transplantation. Cell suspensions (like blood and semen) and thin tissue sections can sometimes be stored almost indefinitely at liquid nitrogen temperature (cryopreservation). Human sperm, eggs and embryos are routinely stored in fertility research and treatments. Controlled-rate and slow freezing are well established techniques pioneered in the early 1970s which enabled the first human embryo frozen birth (Zoe Leyland) in 1984. Since then machines that freeze biological samples using programmable steps, or controlled rates, have been used all over the world for human, animal and cell biology – 'freezing down' a sample to better preserve it for eventual thawing, before it is deep frozen, or cryopreserved, in liquid nitrogen. Such machines are used for freezing oocytes, skin, blood products, embryo, sperm, stem cells and general tissue preservation in hospitals, veterinary practices and research labs. The number of live births from 'slow frozen' frozen embryos is some 300,000 to 400,000 or 20% of the estimated 3 million IVF births. Dr Christopher Chen, Australia, reported the world's first pregnancy using slow frozen oocytes from a British Controlled Rate freezer in 1986.

Cryosurgery (intended and controlled tissue destruction by ice formation) was carried out by James Arnott in 1845 in an operation on a patient with cancer. Cryosurgery is not common.

Preservation techniques

Cryobiology as an applied science is primarily concerned with low temperature preservation. Hypothermic storage is typically above 0°C but below normothermic (32°C to 37°C) mammalian temperatures. Storage by cryopreservation, on the other hand, will be in the −80°C to −196°C temperature range. Organs, and tissues are more frequently the objects of hypothermic storage, whereas single cells have been the most common objects cryopreserved.

A rule of thumb in hypothermic storage is that every 10°C reduction in temperature is accompanied by a 50% decrease in oxygen consumption. Although hibernating animals have adapted mechanisms to avoid metabolic imbalances associated with hypothermia, hypothermic organs and tissues being maintained for transplantation require special preservation solutions to counter acidosis, depressed sodium pump activity and increased intracellular calcium. Special organ preservation solutions such as Viaspan (University of Wisconsin solution), HTK, and Celsior have been designed for this purpose. These solutions also contain ingredients to minimize damage by free radicals, prevent edema, compensate for ATP loss, etc.

Cryopreservation of cells is guided by the "Two-Factor Hypothesis" of American cryobiologist Peter Mazur, which states that excessively rapid cooling kills cells by intracellular ice formation and excessively slow cooling kills cells by either electrolyte toxicity or mechanical crushing. During slow cooling ice forms extracellularly, causing water to osmotically leave cells, thereby dehydrating them. Intracellular ice can be much more damaging than extracellular ice.

For red blood cells the optimum cooling rate is very rapid (nearly 100°C per second), whereas for stem cells the optimum cooling rate is very slow (1°C per minute). Cryoprotectants, such as DMSO (dimethyl sulfoxide) and glycerol, are used to protect cells from freezing. A variety of cell types are protected by 10% DMSO. Cryobiologists attempt to optimize cryoprotectant concentration (minimizing both ice formation and toxicity) as well as cooling rate. Cells may be cooled at an optimum cooling rate to a temperature between −30°C and −40°C before being plunged into liquid nitrogen.

Slow cooling methods rely on the fact that cells contain few nucleating agents, but contain naturally-occurring vitrifying substances that can prevent ice formation in cells that have been moderately dehydrated. Some cryobiologists are seeking mixtures of cryoprotectants for full vitrification (zero ice formation) in preservation of cells, tissues and organs. Vitrification methods pose a challenge in the requirement to search for cryoprotectant mixtures that can minimize toxicity.

Cryobiology in humans

Human gametes and 2, 4 and 8-cell embryos can survive cryopreservation at -196°C for 10 years under well-controlled laboratory conditions.

Cryopreservation in humans with regards to infertility involves preservation of embryos, sperm or oocytes via freezing. Conception, in vitro, is attempted when the sperm is thawed and introduced to the 'fresh' eggs, the frozen eggs are thawed and sperm is placed with the eggs and together they are placed back into the uterus or a frozen embryo is introduced to the uterus. Vitrification has its glitches and is not as reliable or proven as freezing fertilized sperm, eggs or embryos as traditional slow freezing methods because eggs alone are extremely sensitive to temperature. Many researchers are also freezing ovarian tissue in conjunction with the eggs in hopes that the ovarian tissue can be transplanted back into the uterus, stimulating normal ovulation cycles. In Sep 2004 Prof Donnez of Louvain in Belgium reported the first successful ovarian birth from frozen ovarian tissue. In 1997 samples of ovarian cortex were taken from a woman with Hodgkin’s lymphoma and cryo-preserved in a (Planer, UK) controlled rate freezer and then stored in liquid Nitrogen. Chemotherapy was initiated and after the patient had premature ovarian failure. In 2003, after freeze-thawing, orthotopic autotransplantation of ovarian cortical tissue was done by laparoscopy and five months after reimplantation signs indicated recovery of regular ovulatory cycles. Eleven months after re-implantation a viable intrauterine pregnancy was confirmed, which resulted in the first such live birth – a girl called Tamara.

Therapeutic hypothermia, e.g. during heart surgery on a "cold" heart (generated by cold perfusion without any ice formation) allows for much longer operations and improves recovery rates for patients.

Scientific societies

The Society for Cryobiology was founded in 1964 to bring together those from the biological, medical and physical sciences who have a common interest in the effect of low temperatures on biological systems. As of 2007, the Society for Cryobiology had approximately 280 members from around the world, and one half of them are US based. The purpose of the Society is to promote scientific research in low temperature biology, to improve scientific understanding in this field, and to disseminate and apply this knowledge to the benefit of mankind. The Society requires of all its members the highest ethical and scientific standards in the performance of their professional activities. According to the Society's bylaws, membership may be refused to applicants whose conduct is deemed detrimental to the Society; in 1982, the bylaws were amended explicitly to exclude "any practice or application of freezing deceased persons in the anticipation of their reanimation," over the objections of some members who were cryonicists such as Jerry Leaf. The Society organizes an annual scientific meeting dedicated to all aspects of low-temperature biology. This international meeting offers opportunities for presentation and discussion of the most up-to-date research in cryobiology as well as reviewing specific aspects through symposia and workshops. Members are also kept informed of news and forthcoming meetings through the Society newsletter, *News Notes*. The 2009-2010 President of the Society for Cryobiology is Barry J. Fuller.

The Society for Low Temperature Biology was founded in 1964 and became a Registered Charity in 2003 with the purpose of promoting research into the effects of low temperatures on all types of organisms and their constituent cells, tissues and organs. As of 2006, the Society for Low Temperature Biology had approximately 130 (mostly British and European) members and holds at least one Annual General Meeting. The program usually includes both a symposium on a topical subject and a session of free communications on any aspect of low temperature biology. Recent symposia have included long-term stability, preservation of aquatic organisms, cryopreservation of embryos and gametes, preservation of plants, low temperature microscopy, vitrification (glass formation of aqueous systems during cooling), freeze drying and tissue banking. Members are informed through the Society Newsletter, which is presently published 3 times a year. Since 2005, the chair of the Society for Low Temperature Biology has been Tiantian Zhang.

Journals

CRYOBIOLOGY, (publisher: Elsevier) is the foremost scientific publication in this area, with approximately 60 refereed contributions published each year. Articles concern any aspect of low temperature biology and medicine (e.g. freezing, freeze-drying,

hibernation, cold tolerance and adaptation, cryoprotective compounds, medical applications of reduced temperature, cryosurgery, hypothermia, and perfusion of organs).

CRYO LETTERS is an independent UK based rapid communication journal which publishes papers on the effects produced by low temperatures on a wide variety of biophysical and biological processes, or studies involving low temperature techniques in the investigation of biological and ecological topics.

CELL PRESERVATION TECHNOLOGY is a peer-reviewed quarterly scientific journal published by Mary Ann Liebert, Inc. dedicated to the diverse spectrum of preservation technologies including cryopreservation, dry-state (anhydrobiosis), glassy-state and hypothermic maintenance. *Cell Preservation Technology* has been renamed *Biopreservation and Biobanking* and is the official journal of International Society for Biological and Environmental Repositories (ISBER).

Chapter- 6

Cryonically Preserved People

James Bedford

James Hiram Bedford (20 April 1893 – 12 January 1967) was a University of California psychology professor who had written several books on occupational counseling. He is the first person whose body was cryonically preserved (frozen) after legal death, and who remains cryopreserved.

Among those in the cryonics community, the anniversary of his cryonic preservation is celebrated as "Bedford Day".

Cryonic body preservation

In June 1965, Ev Cooper's Life Extension Society (LES) offered to preserve one person free of charge, stating that "the Life Extension Society now has primitive facilities for emergency short term freezing and storing our friend the large homeotherm (man). LES offers to freeze free of charge the first person desirous and in need of cryogenic suspension" and Bedford offered and was accepted as this candidate. Bedford had kidney cancer that had metastasized to his lungs and was untreatable at that time. Bedford also left $100,000 to cryonics research in his will, but more than this amount was spent by Bedford's wife and son defending his will and cryonics suspension wishes in court from claims by other relatives.

Bedford's body was frozen a few hours after he died of natural causes related to his cancer. His body was frozen by Robert Prehoda (author of the 1969 book *Suspended Animation*), Dr. Dante Brunol (physician and biophysicist) and Robert Nelson (President of the Cryonics Society of California). Nelson then wrote a book about the subject titled *We Froze the First Man*. Modern cryonics organizations perfuse cryonics patients with an anti-freeze (cryoprotectant) to prevent ice formation (vitrification), but the use of cryoprotectants in Bedford's case was primitive. He was injected with DMSO, so it is unlikely that his brain was protected. At first, his body was stored at Edward Hope's Cryo-Care facility in Phoenix, Arizona, for two years, then in 1969 moved to the Galiso facility in California. Bedford was moved from Galiso in 1973 to Trans Time near Berkeley, California, until 1977, before being stored by Bedford's son for many years.

Bedford's body was maintained in liquid nitrogen by his family in southern California until 1982, when it was then moved to Alcor Life Extension Foundation, and has remained in Alcor's care to the present day. In May 1991, his body's condition was evaluated when he was moved to a new storage dewar. The examiners concluded that "it seems likely that his external temperature has remained at relatively low subzero temperatures throughout the storage interval."

Personal life

Bedford had a wife, Ruby, a son, Norman, and enjoyed traveling extensively.

Works

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- Bedford, James (1938). *Youth and the world's work: Vocational adjustment of youth in the modern world*. Society for Occupational Research.
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Dick Clair

Dick Clair

	Richard Jones
Born	November 12, 1931 San Francisco, California, United States
Died	December 12, 1988 (aged 57) Los Angeles, California, United States
Years active	1972 - 1987

Dick Clair (November 12, 1931 - December 12, 1988) was an American television producer, actor and television and film writer, best known for the television sitcoms *It's a Living*, *The Facts of Life*, and *Mama's Family*.

Early life

Clair was born **Richard Jones** in San Francisco, California, on November 11, 1931. He served in the military for two years (1955–1957). He never married and never had children.

Career

In the early 1970s Clair performed husband-and-wife comedy routines for *The Ed Sullivan Show* with his partner Jenna McMahon. Clair was a screenwriter for episodes of *The Mary Tyler Moore Show* and *The Bob Newhart Show* in addition to his Emmy Award winning writing for the comedy-variety TV program *The Carol Burnett Show*. With Jenna McMahon he wrote and produced the television sitcoms *It's a Living*, *The Facts of Life*, and *Mama's Family*.

Cryonics involvement

Clair was active as an early member of the Cryonics Society of California in the 1960s. In 1982 he contributed $20,000 to the cryonics organization Trans Time so that a husband and wife could remain cryopreserved in liquid nitrogen. He was diagnosed with AIDS in 1986. When he was hospitalized in 1988 he faced opposition from the hospital and the State of California concerning his desire for cryonics treatment. The ensuing court battle (*Roe v. Mitchell*, with Clair as "John Roe") ended victoriously, establishing the legal right of persons to be cryonically preserved in the state of California.

Death

Clair died on December 12, 1988, of multiple AIDS-related infections at the age of fifty-seven. He was cryopreserved at the Alcor Life Extension Foundation.

Dora Kent

Dora Kent (c. 1904 – December 11, 1987) was the mother of Saul Kent, a board member of Alcor Life Extension Foundation. In her earlier years, Kent worked as a dressmaker in New York. She was the object of a 1988 legal controversy about whether she had been murdered prior to cryonic suspension. She was Alcor's eighth patient and the oldest at that time to ever be cryopreserved.

In December 1987, succumbing to Alzheimer's disease and pneumonia, Kent was brought by her son to the Alcor facility in Riverside, California, where she died. Alcor workers removed her head and stored it in a nitrogen-cooled Dewar flask. No physician was in attendance when she died.

The Riverside County coroner's office, led by Raymond Carrillo, autopsied Kent's headless body and determined the cause of death to be pneumonia. Later, the coroner said that the presence of certain metabolites in the body suggested that she was still alive at the time of preservation. Drugs were used as part of the cryonics process, and it was

therefore difficult to tell whether a drug was administered before or after death. The coroner demanded the head for autopsy, along with all of Alcor's patient records and all its patients' bodies. When Alcor workers refused to produce the head or surrender other patients' bodies, several Alcor workers and volunteers, including Mike Darwin, were handcuffed and arrested, although none were charged.

In a SWAT team raid a week later, most of Alcor's property was seized, although it was later returned. Deputy coroner Dan Cupido said that Alcor had better equipment than some medical facilities. Alcor sued the county for false arrest and illegal seizure and won both suits, including a $90,000 settlement on behalf of the five workers who had been falsely arrested.

Ultimately, the court granted a restraining order against the coroner, protecting the head of Dora Kent and the other frozen human remains at Alcor from seizure, destruction or damage.

The case received much publicity over the ensuing years, which resulted in more interest in Alcor's services and sudden growth in the number of Alcor members.

Jerry Leaf

Jerry D. Leaf (April 4, 1941 – July 10, 1991) was Vice President and Director of the cryonics organization Alcor Life Extension Foundation, and President of the cryonics service firm Cryovita, Inc., until his cryopreservation by Alcor following a fatal heart attack in 1991.

Leaf fought in special operations during the Vietnam War. He also worked as a cardiothoracic surgery researcher at the UCLA School of Medicine, co-authoring more than 20 papers from the laboratory of Dr. Gerald Buckberg.

During the late 1970s and 1980s, Leaf transformed the field of cryonics by bringing unprecedented medical expertise to the field and introducing technologies and procedures of thoracic surgery, especially heart-lung bypass, for improved blood vessel access and life support of cryonics patients. Leaf was involved in the first experiments done by a cryonics organization. He is most famous for developing with Mike Darwin a blood substitute shown capable of sustaining life in dogs for four hours at near-freezing temperatures. Leaf was the head of Alcor's suspension team and participated in many suspensions of Alcor patients.

Cryovita Laboratories

In 1978, after teaching surgery as a research associate at UCLA, Leaf founded Cryovita Laboratories. Cryovita was a for-profit organization which provided cryopreservation services and the building for Alcor in the 1980s, including storage of the first cryonics patient, James Bedford, from 1982. During this time, Leaf also collaborated with Michael Darwin in a series of hypothermia experiments in which dogs were resuscitated with no measurable neurological deficit after hours in deep hypothermia, just a few degrees above zero Celsius. The blood substitute which was developed for these experiments became the basis for the washout solution used at Alcor. Together, Leaf and Darwin developed a standby-transport model for human cryonics cases with the goal of intervening immediately after cardiac arrest and minimizing ischemic injury, the "gold standard" of technology at that time, in which a patient's kidney was considered to be in transplantable condition two days after her death. Leaf and Darwin transferred Bedford, the first person cryopreserved, to a more technologically advanced dewar at Alcor in 1991 and were able to examine him at that time. A member of the Society for Cryobiology, Leaf objected to a 1980s change by the Society to amend its bylaws to prevent cryonicists from holding membership in the Society.

With no history of heart disease, Leaf suffered a fatal heart attack in 1991. Leaf was cryopreserved by Alcor. Today, Alcor is the only full-service cryonics organization that performs remote standbys, using Leaf and Darwin's methods.

Robert Ettinger

Robert Chester Wilson Ettinger (born December 4, 1918) is known as "the father of cryonics" due to the impact of his 1962 book *The Prospect of Immortality*. He is considered by some a pioneer transhumanist on the basis of his 1972 book *Man into Superman*.

Ettinger founded the Cryonics Institute and the related Immortalist Society and until 2003 served as the groups' president. His first and second wives have both been cryopreserved as well as his mother.

Personal background

Ettinger served as a second lieutenant infantryman in the United States Army during World War II. Severely wounded in battle in Germany, he received the Purple Heart and recovered after several years spent in a Michigan hospital. He earned two Master's degrees from Wayne State University (one in physics, one in mathematics) and spent his working career teaching physics and mathematics at both Wayne State University and Highland Park Community College in Michigan.

Ettinger had two children with his first wife, Elaine, David (1951) and Shelley (1954). David gave his first cryonics interview to journalists at the age of 12 and has been active in the field ever since. David Ettinger is an attorney, and he currently serves as legal counsel to the Cryonics Institute and the Immortalist Society. Robert Ettinger's daughter has had no interest in cryonics.

Ettinger met his second wife, Mae Junod, in 1962 when she attended one of his adult education courses in basic physics. Junod typed and assisted with editing the manuscripts for both *The Prospect of Immortality* and *Man into Superman*. She became active in the Cryonics Society of Michigan (CSM) and edited and was production manager for the CSM monthly newsletter, *The Outlook*. In the 1970s *The Outlook* was renamed *The Immortalist* and Junod continued editorship until the mid-1990s. *The Outlook* is the longest continuously published cryonics magazine. Junod was an author, feminist, and marriage counselor.

Ettinger married Junod in 1988 after the death of his first wife. Ettinger described his time with Junod as one of the most satisfying and tranquil times in his life. The couple moved to Scottsdale, Arizona in 1995 and enjoyed a period of domestic life during which time the couple began to ease into retirement from over 30 years of cryonics activism and the attendant burdens of work and controversy. Mae Ettinger suffered a debilitating stroke in 1998 from which she never fully recovered followed by a lethal stroke in 2000, which resulted in her cryopreservation.

Roots of cryonics in science fiction

Ettinger grew up reading Hugo Gernsback's *Amazing Stories*. Ettinger was particularly affected when he was 12 years old by a Neil R. Jones story, "The Jameson Satellite," which appeared in the July 1931 issue of *Amazing Stories*, in which one Professor Jameson had his corpse sent into earth orbit where (as the author mistakenly thought) it would remain preserved indefinitely at near absolute zero. And so it did, in the story, until millions of years later, when, with humanity extinct, a race of mechanical men with organic brains chanced upon it. They revived and repaired Jameson's brain, installed it in a mechanical body, and he became one of their company.

Ettinger assumed that one day— long before he grew old-- biologists would learn the secret of eternal youth. As he grew out of boyhood in the 1930s, he began to suspect it might take a little longer since no scientists were yet working on this particular endeavor. If immortality is achievable through the ministrations of technologically advanced aliens repairing a frozen human corpse, then Ettinger thought everyone could be cryopreserved to await later rescue by our own medically more sophisticated descendants.

In 1947 while in the hospital for his battle wounds, Ettinger discovered that research in the area of cryogenics was being done by French biologist Jean Rostand; Ettinger wrote a short story elucidating the concept of human cryopreservation as a pathway to more sophisticated future medical technology: in effect, a form of one-way medical time travel. The story, "The Penultimate Trump," was published in the March 1948 issue of *Startling*

Stories and definitively establishes Ettinger's priority as the first person to have promulgated the cryonics paradigm, principally that contemporary medical/legal definitions of death are relative, not absolute, and are critically dependent upon the sophistication of available medical technology. Thus, a person apparently dead of a heart attack in a tribal village in the Amazon will soon become unequivocally so, whereas the same person with the same condition in the emergency department of large, industrialized city's hospital, might well be resuscitated and continue a long and healthy life. Ettinger observed that criteria for death will vary not just from place to place, but from time to time, and so today's corpse could be tomorrow's patient.

Launching the cryonics movement

Ettinger waited expectantly for prominent scientists or physicians to come to the same conclusion he had, and to take a position of public advocacy. By 1960, Ettinger finally made the scientific case for the idea, which had always been in the back of his mind. Ettinger was 42 years old and said he was increasingly aware of his own mortality. In what has been characterized as an historically important mid-life crisis, Ettinger summarized the idea of cryonics in a few pages, with the emphasis on life insurance, and sent this to approximately 200 people whom he selected from *Who's Who in America*. The response was very small, and it was clear that a much longer exposition was needed— mostly to counter cultural bias. Ettinger correctly saw that people, even the intellectually, financially and socially distinguished, would have to be educated into understanding his belief that dying is usually gradual and could be a reversible process, and that freezing damage is so limited (even though fatal by present criteria) that its reversibility demands relatively little in future progress. Ettinger soon made an even more troubling discovery, principally that "a great many people have to be coaxed into admitting that life is better than death, healthy is better than sick, smart is better than stupid, and immortality might be worth the trouble!"

In 1962, Ettinger privately published a preliminary version of *The Prospect of Immortality*, in which he said that future technological advances could be used to bring people back to life. This finally attracted attention of a major publisher, which sent a copy to Isaac Asimov; Asimov said that the science behind cryonics was sound, and the manuscript was approved for a 1964 Doubleday hardcover and various subsequent editions which launched cryonics. The book became a selection of the Book of the Month Club and was published in nine languages.

Ettinger became an "overnight" media celebrity, discussed in *The New York Times*, *Time*, *Newsweek*, *Paris Match*, *Der Spiegel*, *Christian Century*, and dozens of other periodicals. He appeared on television with David Frost, Johnny Carson, Steve Allen, and others. Ettinger also spoke on radio programs coast-to-coast to promote the idea of human cryopreservation.

Since the commercial publication of *The Prospect of Immortality*, all those active in cryonics today can trace their involvement, directly or indirectly, to the publication of one or both of Ettinger's books. While Ettinger was the first, most articulate, and most

scientifically credible person to argue the idea of cryonics, he was not the only one. In 1962, Evan Cooper had authored a manuscript entitled *Immortality, Scientifically, Physically, Now* under the pseudonym "N. Durhing". Cooper's book contained the same argument as did Ettinger's, but it lacked both scientific and technical rigor and was not of publication quality.

Organizational activities

Following publication of *The Prospect of Immortality*, Ettinger again waited for prominent scientists, industrialists, or others in authority to see the wisdom of his idea and begin implementing it. By contrast, Cooper was an activist and must be credited with forming the first cryonics organization (although the word "cryonics" was not to be coined until 1965) the Life Extension Society (LES). LES advocated immediate action to implement human cryopreservation and established a nationwide network of chapters and coordinators to develop a grassroots capability for delivering cryopreservation on an emergent basis. Cooper left cryonics activism in 1969, and was lost at sea in 1983. But his activities with LES provided the basis for the formation of the first Cryonics Societies.

In 1966 the Cryonics Societies of California and Michigan were formed. Ettinger was elected President of the Cryonics Society of Michigan (CSM). In 1970s CSM was transformed under the direction of Ettinger into the Cryonics Institute (CI) and the Immortalist Society (IS). In 1976, Ettinger's mother, Rhea Ettinger, became CI's first patient. Ettinger was President of both CI and IS until 2003.

From 1964 until circa 1990 the growth of the cryonics movement was slow. During this period cryonicists suffered from lack of consistent or quality professional medical, legal, philosophical, business or financial support. Admission of interest in, or advocacy of cryopreservation, uniformly resulted in reactions of revulsion, ridicule, or both. Media and public perception were consistently negative. This external pressure was exacerbated by the anxiety and fear felt as cryonicists experienced the death of cohorts and loved ones and were, of necessity, forced to provide whatever level of care they could manage on a more or less mutual aid basis. Cryonics, contrary to public perception at this time, was (and still is) a middle class undertaking, and the resources available were those of mortuary personnel and equipment and procedures which cryonicists were able to construct and devise themselves. An additional worry was the uncertain legal status of cryonics and the ever present possibility of governmental interdiction.

The growth of the internet has made a crucial difference to the spread of the cryonics meme (idea), which, despite much media coverage, seems to be mainly dependent upon personal contact and personal investigation.

Quotes by Ettinger

"I had and have, no credentials worth mentioning being only a teacher of college physics and math. It is precisely this that prevented me, for so long, from doing more: I knew I carried no weight, had no formal qualifications, and was not suited for a leadership role. But as the years passed and no one better came forward, I finally had to write, and later felt I had to form organizations (although others had come into existence). This tragedy, in various manifestations, may persist. Potentially effective leaders may have turned aside because I (and later a few other obscure people) reluctantly preempted leadership. Business people and investors may have hesitated because the small, poorly capitalized organizations already in the field have had such limited (although increasing!) success in attracting participants."

"Tragedy is in the eye of the beholder. As Sid Caesar (or maybe Mel Brooks -- one of those really heavy thinkers) said: 'The difference between comedy and tragedy? When the saber tooth tiger eats Moe, that's comedy. When I get a hangnail, that's tragedy.' And if the Tiger of Death eats you, that is the ultimate tragedy; that is when the world ends, when the cosmos disappears, when Everything becomes Nothing.

"The 'tragedy' of the slow growth of immortalism pertains mostly to them, and perhaps to you -- not so much to me or to us, the committed immortalists. We already have made our arrangements for cryostasis after clinical death -- signed our contracts with existing organizations and allocated the money. We will have our chance, and with a little bit of luck will 'taste the wine of centuries unborn'. "

Chapter- 7

Alcor Life Extension Foundation

Alcor Life Extension Foundation

Founders	Fred & Linda Chamberlain
Founded	1972
Location	Scottsdale, Arizona 33°37′4″N 111°55′30″W / 33.61778°N 111.925°W
Area served	Global
Focus	cryonics
Method	Application and further development of cryonics. Education of the public about cryonics.
Revenue	Membership fees and donations; The Alcor Patient Care Trust
Employees	12
Members	932

This "bigfoot" Dewar is custom-designed to contain four wholebody patients and six neuropatients immersed in liquid nitrogen at −196 degrees Celsius. The Dewar is an insulated container which consumes no electric power. Liquid nitrogen is added periodically to replace the small amount that evaporates.

The **Alcor Life Extension Foundation** is a Scottsdale, Arizona, USA-based nonprofit company that researches, advocates for and performs cryonics, the preservation of humans in liquid nitrogen after legal death, with hopes of restoring them to full health when new technology is developed in the future.

As of December 31, 2010, Alcor had 932 members, and 102 patients in cryopreservation, many as neuropatients (67 of Alcor patients were neuropatients as of December 2010).

Alcor accepts anatomical donations (cryonics cases) under the Uniform Anatomical Gift Act and Arizona Anatomical Gift Act for research purposes, reinforced by a court case in its favor that affirmed a constitutional right to engage in cryopreservation and donate one's body for the purpose. A form of the Uniform Anatomical Gift Act has been passed in all 50 states.

History

The largest cryonics organization today, in terms of membership, was established as a nonprofit organization by Fred and Linda Chamberlain in California in 1972 as the Alcor Society for Solid State Hypothermia (ALCOR). Alcor was named after a faint star in the Big Dipper. The name was changed to Alcor Life Extension Foundation in 1977. The organization was conceived as a rational, technology-oriented cryonics organization that would be managed on a fiscally conservative basis. Alcor advertised in direct mailings and offered seminars in order to attract members and bring attention to the cryonics movement. The first of these seminars attracted 30 people.

On July 16, 1976, Alcor performed its first human cryopreservation on Fred Chamberlain's father. That same year, research in cryonics began with initial funding provided by the Manrise Corporation. At that time, Alcor’s office consisted of a mobile surgical unit in a large van. Trans Time, Inc., a cryonics organization in the San Francisco Bay area, provided initial preservation procedures and long-term patient storage until Alcor began doing its own storage in 1982.

In 1977, articles of incorporation were filed in Indianapolis by the Institute for Advanced Biological Studies (IABS) and Soma, Inc. IABS was a nonprofit research startup led by a young cryonics enthusiast named Steve Bridge, while Soma was intended as a for-profit organization to provide cryopreservation and human storage services. Its president, Mike Darwin, subsequently became a president of Alcor. Bridge filled the same position many years later. IABS and Soma relocated to California in 1981. Soma was disbanded, while IABS merged with Alcor in 1982.

In 1978, Cryovita Laboratories was founded by Jerry Leaf, who had been teaching surgery at UCLA. Cryovita was a for-profit organization which provided cryopreservation and transport services for Alcor in the 1980s until Leaf's death, at which time Alcor began providing these services on its own. Leaf and Michael Darwin collaborated to bring the first cryonics patient, Dr. James Bedford, who was preserved in 1967, to Alcor's California facility in 1982.

During this time, Leaf also collaborated with Michael Darwin in a series of hypothermia experiments in which dogs were resuscitated with no measurable neurological deficit after hours in deep hypothermia, just a few degrees above zero Celsius. The blood substitute which was developed for these experiments became the basis for the washout solution used at Alcor. Together, Leaf and Darwin developed a standby-transport model for human cryonics cases with the goal of intervening immediately after cardiac arrest and minimizing ischemic injury. Leaf was cryopreserved by Alcor in 1991; since 1992,

Alcor has provided its own cryopreservation as well as patient-storage services. Today, Alcor is the only full-service cryonics organization that performs remote standbys.

Alcor grew slowly in its early years. In 1984, it merged with the Cryonics Society of South Florida. Alcor counted only 50 members in 1985, which was the year it cryopreserved its third patient. However, during this time researchers associated with Alcor contributed some of the most important techniques related to cryopreservation, eventually leading to today's method of vitrification.

Increasing growth in membership during this period is partially attributed to the 1986 publication of Eric Drexler's *Engines of Creation*, which debuted the idea of nanotechnology and contained a chapter on cryonics. In 1986, a group of Alcor members formed Symbex, a small investment company which funded a building in Riverside, California, for lease by Alcor. Alcor moved from Fullerton, California, to the new building in Riverside in 1987; Timothy Leary appeared at the grand opening. Alcor cryopreserved a member's companion animal in 1986, and two people in 1987. Three human cases were handled in 1988, including the first whole body patient of Alcor's, and one in 1989. At that time, Alcor owned 20% interest in Symbex, with a goal of 51% ownership. In September 1988, Leary announced that he had signed up with Alcor, becoming the first celebrity to become an Alcor member. Leary later switched to a different cryonics organization, CryoCare, and then changed his mind altogether. Alcor's Vice-President, Director, head of suspension team and chief surgeon, Jerry Leaf, died suddenly of a heart attack in 1991.

By 1990, Alcor had grown to 300 members and outgrown its California headquarters, which was the largest cryonics facility in the world. The organization wanted to remain in Riverside County, but in response to concerns that the California facility was also vulnerable to earthquake risk, the organization purchased a building in Scottsdale, Arizona in 1993 and moved its patients to it in 1994.

Alcor has held seven conferences on life extension technologies, with speakers such as Eric Drexler, Ralph Merkle, Ray Kurzweil, Aubrey de Grey, Timothy Leary, and Michael D. West.

Research

In 2001, Alcor adapted cryoprotectant formulas from published scientific literature into a more concentrated formula capable of achieving ice-free preservation (vitrification) of the human brain (neurovitrification). In 2005, the vitrification process was applied to the first whole-body subject (as opposed to brain-only). This resulted in vitrification of the brain and conventional cryopreservation of the rest of the body. Work is continuing towards achieving whole-body vitrification, which is limited by the ability to fully circulate the cryoprotectant throughout the body. The vitrification used since 2000 was switched to what Alcor said was a superior solution in 2005. Canadian businessman Robert Miller, founder of Future Electronics, has provided research funding to Alcor in the past.

Policies and procedures

Alcor is governed by a self-perpetuating board of directors. Alcor's Scientific Advisory Board currently consists of Antonei Csoka, Aubrey de Grey, Robert Freitas, Bart Kosko, James B. Lewis, Ralph Merkle, Marvin Minsky, Martine Rothblatt, and Michael D. West. Alcor also maintains a medical advisory board consisting of medical doctors.

Most Alcor patients fund the procedure through life insurance policies which name Alcor as the beneficiary. Members who have signed up wear medical alert bracelets informing hospitals and doctors to notify Alcor in case of any emergency; in the case of a person who is known to be near death, Alcor can send a team for remote standby.

In some states, members can sign certificates stating that they wish to decline an autopsy. The cutting of the body organs (especially the brain) and blood vessels required for an autopsy makes it difficult to either preserve the body, especially the brain, without damage or perfuse the body with glycerol. The optimum preservation procedure begins less than one hour after death. Members can specify whether they wish Alcor to attempt to preserve even if an autopsy occurs, or whether they wish to be buried or cremated if an autopsy renders little hope for preservation.

In cases with remote standby, cardiopulmonary support is begun as soon as a patient is declared legally dead. Some patients were not able to receive cardiopulmonary support immediately, but in deference to the possibilities of future technology, these patients have also been preserved with the best techniques available. Alcor has a network of paramedics nationwide and seven surgeons, located in different regions, who are on call 24 hours a day. If an Alcor patient is met by a standby team (usually at a hospital, hospice, or home), the team will perform CPR to maintain blood flow to the brain and organs while simultaneously pumping an organ preservation solution through the veins.

Patients are transported as quickly as possible to Alcor headquarters in Scottsdale, where they undergo final preparations in Alcor's cardiopulmonary bypass lab. Plans are underway for a second operating room to be built. In the Patient Care Bay, patients are monitored by computer sensors while kept in liquid nitrogen in dewars. Liquid nitrogen is refilled on a weekly basis and does not need electricity to operate. Riverside County, California deputy coroner Dan Cupido said that Alcor had better equipment than some medical facilities.

Membership dues cover one-third of Alcor's yearly budget, with donations covering the rest. Alcor receives $50,000 each year from television royalties donated by a sitcom writer and producer who is in suspension. In 1997, after a substantial effort led by then-president Steve Bridge, Alcor formed the Patient Care Trust as an entirely separate entity to manage and protect the funding for cryopatients, including owning the building. Alcor remains the only cryonics organization to segregate and protect patient funding in this way; the 2% annual growth of the Trust is enough for upkeep of the patients. At least $70,000 of the money received for each full-body patient goes into this trust for future patient care, $17,000 for a neuropatient. Alcor is currently working to create an Alcor

Model Trust, which would make it easier for members to establish their own Trusts to preserve their assets following legal death and prior to being revived from cryopreservation. Some members have already taken steps to do this on their own. Members can also store possessions deep underground in a Kansas salt mine operated by Underground Vaults & Storage, Inc.

Membership

Members suspended include Dick Clair, an Emmy Award-winning television sitcom writer and producer, Hall of Fame baseball legend Ted Williams and his son John Henry Williams, and futurist FM-2030. Current members of Alcor include nanotechnology pioneer Eric Drexler, Internet pioneer Ralph Merkle, engineer Keith Henson and his family, MIT professor Marvin Minsky, aging researcher Aubrey de Grey, mathemetician Edward O. Thorp, computer security CEO Kenneth Weiss, casino owner Don Laughlin, inventor Ray Kurzweil, film director Charles Matthau, futurists Max More and Natasha Vita-More, entrepreneurs Saul Kent, Luke Nosek and Future Electronics founder Robert Miller. Magazine publisher Althea Flynt was signed up to Alcor, but her body was not able to be preserved after her death, which required an autopsy. One Alcor member died in the World Trade Center in the September 11 attacks.

Membership has grown at a rate of about eight percent a year since Alcor's inception, tripling between 1987 and 1990. The oldest patient at Alcor is a 101-year-old woman, and the youngest is a 18-year-old woman. Alcor has had patients from as far as Australia. One in four of its members resides in the San Francisco Bay Area.

The membership receives Alcor's magazine, *Cryonics*, published four times a year. Keith Henson wrote a column in *Cryonics* for a few years.

Controversies

Dora Kent

Before the company moved to Arizona from Riverside, California in 1994, it became a center of controversy when a county coroner ruled that Alcor client Dora Kent (Alcor board member Saul Kent's mother) was murdered with barbiturates before her head was removed for neuropreservation by the company's staff. Alcor contended that the drug was administered after her death. No charges were ever filed; former Riverside County deputy coroner Alan Kunzman later claimed that this was due to mistakes and poor decision-making by others in his office.

A judge ruled that Kent was already deceased at the time of preservation, and no foul play was involved. Alcor sued the county for false arrest and illegal seizure and won both suits. The incident is credited with spurring a growth in membership for Alcor due to the resultant publicity.

Ted Williams

In 2002, Alcor drew considerable attention when baseball star Ted Williams was placed in cryonic suspension; although Alcor maintains privacy of its patients if they wish and did not disclose that Williams was at the Scottsdale facility, the situation came to light in court documents that grew out of an extended family dispute over Williams' wishes in regard to his remains. While Williams' children Claudia and John Henry contended that Williams wished to be preserved at Alcor, their half-sister and oldest Williams child Bobby-Jo Ferrell contested that her father wished to be cremated. Williams' attorney produced a note signed by Williams, John Henry, and Claudia saying: "JHW, Claudia and Dad all agree to be put into biostasis after we die. This is what we want, to be able to be together in the future, even if it is only a chance." John Henry later said, "He was very into science and believed in new technology and human advancement and was a pioneer. Even though things seemed impossible at times, he always knew there was always a chance to catch a fish -- only if you had your fly in the water."

In 2003, *Sports Illustrated* published allegations by former Alcor COO Larry Johnson that the company had mishandled Williams' head by drilling holes and accidentally cracking it. Johnson also claimed that some of Williams' DNA was missing; the article alleges that Williams' son, John Henry Williams, desired to sell some of his father's DNA, a charge John Henry denied. Williams' attorney called the DNA allegations an "absurd proposition" and accused Johnson of trying to grab headlines. Alcor denied the allegations of missing DNA and explained that microscopic cracking can result as part of the process of freezing the head, damage which is less than previous methods using glycerol during cryopreservation; Alcor believes that technology sufficient to revive its patients would also be able to repair the microscopic fractures, which are monitored using a tiny microphone. In the wake of the *Sports Illustrated* story, Johnson began a paid-membership website where he displayed what he said were photographs of Williams.

John Henry Williams subsequently died of leukemia, and his remains are also stored at Alcor. After John Henry's death, Ferrell again filed a lawsuit, but representatives of Williams' estate repeated that he wished to be at Alcor.

1992 death

In addition to his Williams allegations, Johnson handed over to the police a taped conversation in which he claims Alcor facilities engineer Hugh Hixon stated that an Alcor employee deliberately hastened the imminent 1992 death of a terminally ill AIDS patient, with an injection of Metubine, a paralytic drug. The nurse who pronounced the 1992 death has denied Johnson's claim that there was any hastening of death. The nurse's claim that the patient died in his bedroom contradicts Alcor's own 1992 case report, in which they state the patient died approximately 30 minutes after they transported him to a makeshift operating room, in a garage. In 2009, Carlos Mondragon, (Alcor's CEO at the time of the incident), told ABC News he had been made aware of the allegations, at the time of the case, and as a result, had severed Alcor's ties with the employee who allegedly hastened the patient's death. Mr. Mondragon failed to inform ABC News that

the same person later performed Alcor's surgical procedures, including the neurosuspension of Ted Williams.