Photography by Howard Cao

Life and Death at Low Temperatures

How to freeze and revive a garden snail. By Charles Platt

Few people realize that the processes of life can restart after long periods in which they are ceased completely. Any backyard biologist can verify this strange fact by performing a couple of experiments that originated in the work of a solitary scientist named Basile Luyet, more than half a century ago.

Luyet was a Swiss Jesuit priest who immigrated to the United States in 1929, did graduate work at Yale, and then set up his own small laboratory in Madison, Wisc., where he toiled in isolation for many years.

He wanted to preserve living creatures in such a way that they could be revived at any time in the future. He knew that this was possible, since some creatures can return from days or even years of lifelessness.

The tardigrade, for instance, is a kind of worm about one millimeter long that is able to withstand temperatures down to almost absolute zero.

Reducing temperature is a logical way to preserve life, because living things are composed of cells, and chemical reactions inside cells occur more slowly when they are deprived of heat.

Even human beings can withstand a moderate amount of cooling. To repair a brain aneurysm, surgeons may reduce normal body temperature by more than 20 degrees Centigrade (36 degrees Fahrenheit), which slows the metabolic rate so that a patient may remain in a state of circulatory arrest for as long as one hour. Brain activity ceases during this period and resumes when the patient's normal body temperature is restored.

THE PROBLEM WITH FREEZING CELLS

However, the freezing point of water forms a barrier below which most mammals cannot go. Ice causes catastrophic damage, although not because it "bursts" cells, as is commonly believed.

When the water inside cells freezes, it forms rigid hydrogen-oxygen bonds that exclude other molecules, such as the sodium and potassium salts that are essential for cellular functions. As the ice accumulates, the normally beneficial impurities are squeezed out and form toxic concentrations that literally poison the cell.

For a graphic demonstration of this phenomenon, place some apple juice in a freezer until more than half of it has turned solid. Now take a sip of the remaining liquid, and you'll find it is the most intense juice you have ever tasted. The water that was mixed with the juice has extracted itself as pure ice, leaving only concentrated juice.

Luyet was well aware of the damage caused when ice forms in cells. He tried to avoid it by freezing tiny tissue samples very rapidly, so that ice molecules would not have time to organize themselves in a crystal structure. As he explained to a visiting journalist, "Living matter can survive freezing, but only if the molecules are not ordered, but solidified where they are ... in disordered or uncrystallized form."

Unfortunately, this technique cannot be used on larger groups of cells, because there is no way to cool them fast enough. In the 1950s, three British scientists who were familiar with Luyet's work solved the dilemma by using glycerol, a form of antifreeze, to replace cellular water and reduce the volume of ice. They described the glycerol as a "cryoprotectant" and named their new field of research "cryobiology," from the Greek word "kryos," meaning "cold." Today, glycerol is routinely used to protect sperm and ova that are preserved in liquid nitrogen. Countless babies have been born from human germ plasm that has endured long periods of lifeless storage with this method.

SAFELY FREEZING AND THAWING AN ORGAN

Human organs represent a bigger challenge because their intricate, delicate structure is easily disrupted by ice crystallization. Luyet managed to revive some fragments of rat hearts, but never recovered a whole organ from a very low temperature. That achievement eluded scientists until 2005, when a team led by cryobiologist Gregory Fahy successfully reimplanted a rabbit kidney after it had been preserved at -130°C. Dr. Fahy spent a large part of his professional life perfecting the cryoprotectant that enabled this feat, which requires a well-equipped laboratory. Still, Fahy points out that backyard cryobiologists can still replicate the very simple demos that Basile Luyet ran more than 50 years ago.

The simplest one doesn't even require glycerol. "I've been told that Luyet would toss two goldfish into some liquid nitrogen," Fahy says. "He would quickly withdraw Goldfish A, which would seem stiff and frozen — but it would resume wriggling after it was placed in warm water."

Luyet would then startle onlookers by removing Goldfish B and snapping it in half. This may not have been a completely fair demonstration, since Goldfish B was exposed to liquid nitrogen a little longer than Goldfish A. It's not clear whether Luyet realized that, to some extent, he had rigged his experiment, but at the very least it remains a unique ... icebreaker?

Liquid nitrogen is available in most urban areas (search online for "liquid gases"), and it is generally inexpensive. It is nontoxic, but must be handled with caution, since its temperature of -196°C can cause serious injury to any exposed human tissue. Always wear heavy gloves and eye protection!

PUTTING A SNAIL INTO SUSPENDED ANIMATION

If you live in a part of the world where snails are abundant, Fahy suggests another simple experiment, which he performed himself as a high school student. All you need is a domestic freezer and a thin-walled vial, such as the kind used for prescription medications. First, catch your snail. This is the easy part. Now place the snail in the container, top it off with water, and put it in your freezer. Wait just long enough for the water to turn to ice (an hour should be sufficient). Quickly remove the container and run it under warm water. As the ice melts, transfer your snail to a smooth surface such as a dinner plate, and continue the warm-water treatment.

When the snail recovers its senses, it should start crawling away at a fast pace (relatively speaking). If your snail turns yellow and breaks in half, you left it in the freezer too long. Even snails become injured when they are 100% frozen without protection from ice damage.

Those who want to take cryobiology more seriously can obtain some glycerol from a chemical supply company. (Glycerol is nonhazardous as long as you refrain from drinking it.) If you have access to a cheap microscope, you can replicate British research from the 1950s by soaking sperm or blood cells in various concentrations of glycerol. Subject your samples to liquid nitrogen, then rewarm them and see if the cells resume their activity.

If you're feeling even more ambitious, you can perform the same procedure with nematode worms, which are as small as tardigrades but more readily available. In fact, your backyard may be crawling with them. You can also buy them by mail order from ecologically enlightened companies that sell them as a natural pest control, since the microscopic worms eat fly larvae. Reviving a nematode from a period in liquid nitrogen is a challenge, but it can be done.

Basile Luyet never achieved his ambition to stop and start life processes on a large scale, but his basic studies had serious long-term implications.

Clearly, a surgical patient who reawakens after zero brain activity at a low temperature has not been dead in the usual meaning of the term. By the same logic, a frozen blood cell, or a cryopreserved nematode, is not dead either. If we can revive a rabbit kidney, or (eventually) a whole animal, by preserving it in such a way that its cells remain viable, we may begin to challenge the conventional concept of death to the point where it becomes virtually meaningless.

FURTHER READING

societyforcryobiology.org
21cm.com
alcor.org

Charles Platt has been a senior writer for *Wired* and has written science fiction novels such as *The Silicon Man*.