Frozen Frog May Give Docs Jump on Human Transplants

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The common wood frog displays a rare trait called freeze tolerance. When the mercury falls, the animal becomes, to the eye and touch, a frog- shaped ice cube. The way it does this may eventually be copied to aid human organ transplants.

"Two-thirds of their body water, or more, freezes," explained Jack Layne, a biologist at Slippery Rock University of Pennsylvania. "The heart stops, the breathing stops. For all practical purposes you'd assume that it was dead."

In reality, the frog's metabolism slows to a crawl, and its body temperature drops to between 21° and 30° Fahrenheit (–6° and –1° Celsius). The amphibian's heart and brain cease to function.

Frozen frog experts, such as biochemists Ken and Janet Storey of Carleton University in Ottawa, Canada, believe the animals acquired their ability to withstand a deep freeze about 15,000 years ago, during ice age evolution.

Freeze tolerance allows common wood frogs *(Rana sylvatica)* to live in harsh climates as far north as the Arctic Circle—the only frogs to do so. But they can also be found as far south in the United States as Georgia.

The amphibians cannot survive if their body temperature drops below about 20° Fahrenheit (–6° Celsius). But snow pack and other natural insulators can keep the frogs sufficiently warm during their winter hibernation.

A key to their survival is a natural antifreeze that prevents the amphibians' cells from dehydrating excessively during the freezing process.

Frozen Brains, Hearts

During this process, about two-thirds of the frog's body water freezes. The remainder, including water inside cells, remains liquid. Glucose produced by the amphibian's liver lowers the tissue freezing point (in the same way that ammonia lowers the freezing temperature of a car's windshield wiper fluid, which is mostly water.)

The glucose limits ice formation in the body and binds water molecules within the frog's cells. This curbs the damage caused by cell shrinkage, which is common with freezing.

"Normally under those freezing conditions, without glucose, the cells would dehydrate completely," said Boris Rubinsky, an engineer at the University of California at Berkeley.

In a recent issue of *Discover* magazine Rubinsky published images of temperature scanning electron micrographs (a sort of heat-based CT scan) of frozen common wood frogs. A cross section of a frog's liver illustrates how water remains in the cells.

Rubinsky said he has used other imaging technology to study the frogs.

"We've done MRIs of frogs. And without harming the animal, we were able to observe the entire process of [the] freezing of a live animal as it happens in nature," he said.

"When it thaws, it thaws from the interior out. … [T]he heart begins to thaw first and then the brain and, only at the end, the limbs."

In nature and in the lab, the thawing process takes places of a period of several hours. When complete, the amphibians restart their hearts and hop away unscathed.

The process is remarkable but not unique.

"There are a number of insect species whose body fluids will freeze quite substantially," said Layne, the Slippery Rock University biologist. He added that about six North American frog species, one European lizard, and a handful of North American turtles also withstand deep-freezing. He suspects the ability may also one day be observed in a number of Asian animal species.

Other animals use a variety of mechanisms to deal with cold, from hibernation to migration. So why do frogs freeze?

Layne believes that in the case of the wood frogs, freezing is somehow related to reproductive strategy.

"Most freeze-tolerant [species] like to breed early in the spring," he said. "They lay eggs in ephemeral ponds that develop from snow melt. [The frogs] have to get into those ponds quickly when they get a good melt, because [the ponds] dry up in summer, and that could result in a lost generation."

Frogs in nonfrozen deep hibernation, such as on a pond bottom, take longer to emerge from hibernation to answer the call of the spring mating season.

While common wood frogs put their freeze tolerance to good use, many scientists are pondering what use humans might make of the process.

Better Organ Transplants?

"These frogs and turtles are vertebrate animals and share a lot of things in common with mammals, like organ and tissue structures," explained Jon Costanzo from the Laboratory for Ecophysiological Cryobiology at Miami University in Oxford, Ohio. "It begs the question: If a frog can withstand the freezing of all of its organs at the same time, how can we apply that to humans?"

One avenue of particular interest to Rubinsky, the Berkeley engineer, and other researchers is the field of organ transplants.

"Fifty thousand people in the United States are waiting for a heart transplant, but only about 3,000 will get one," Rubinsky said.

Time is a major constraint. In the case of a human heart transplant, doctors have only five or six hours from the time the donor organ is harvested until it must be implanted in the living recipient.

The logistics of quickly moving a matched organ from donor to recipient—who are often separated by considerable distance—make many transplants impossible. But slowing the metabolism of the donated organ via techniques like freezing is a possible solution.

"It's the kind of problem in which small improvements could make a big difference," Rubinsky noted. "For instance, having 24 hours instead of 6 could provide a lot more organs to a lot more people."

Rubinsky has had some success. In 1999 he and colleagues preserved rat livers in a partially frozen state, then thawed and implanted the organs in "recipient" rats—one of which survived for five days.

More recently Rubinsky and colleagues at Sheba Medical Center in Israel have made some progress in freezing rat hearts.

But the road ahead will be a difficult one. "When it comes to more complicated structures [researchers] just haven't been making much progress," said Costanzo, the Miami University researcher. He believes science would benefit by trying to more closely mimic nature.

"I think, in the past, people used techniques that were very different from what the animals are doing," he said. "The animals are cooling very slowly, and the temperature they remain at is really quite high. Just a few degrees below 0° Celsius [32° Fahrenheit]."

The cryoprotectants (additives used to preserve frozen tissue) now routinely used for embryo and sperm preservation were unheard-of a half century or so ago. So who knows where future research may lead?

Noting the common wood frog, Layne, the Slippery Rock University biologist, said, "You've got an animal here that experiences total cardiac arrest. Its heart stops. When it comes to things like a [human] stroke, this parallels that in a way. You have a cessation of blood flow and then it starts again.

"There's always a medical debate about restoring blood flow after blockage," he said. "These frogs start everything back up, and they don't have the injury. The model might [give] some insight when it comes to conditions like heart attack and stroke."

Of course, some eye the frogs and ponder the ultimate human application—life preservation by freezing entire bodies of the deceased. While no door is closed, most scientists agree that success in this arena, if possible, is many years down the road.

As Layne noted, "Even nature has not evolved a freeze-tolerant mammal."

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