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ICE, THE CRADLE OF LIFE

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In 1953, Stanley Miller first created the building blocks of life in his laboratory by simulating lightning on early Earth. His final experiment, 45 years later, has opened a surprising new chapter in the search for life's origins.

One morning in late 1997, Stanley Miller lifted a glass vial from a bubbling vat. For 25 years he had cared for that vial as though it were an exotic orchid, checking on it each morning, adding a few pellets of dry ice to keep it frozen, at –108 ºF. He had told hardly a soul of its existence.

With wrinkled, 70-year-old hands, Miller finally laid his frozen time capsule down to thaw on the counter—ending the experiment that had lasted a third of his life. The contents of that vial would arouse both surprise and skepticism around the world.

Miller had filled the vial in 1972 with a primordial mix of ammonia and cyanide. He then cooled it to the temperature of Jupiter's icy moon Europa—too cold, most scientists would assume, for much to happen.

But as Miller and his former student Jeffrey Bada brushed the frost from the vial that morning, they could already see that something had happened. The mixture of ammonia and cyanide—normally colorless—had deepened into yellow-brown, highlighting a web of cracks in the ice.

Tests later confirmed their hunch—that in 25 years the frozen mixture had coalesced into the molecules of life: building blocks of RNA, DNA, and proteins. The results of that experiment would tilt the tables of how we think that life began on Earth.

For decades, people studying the origin of life have imagined primordial soups, tropical ponds—even boiling volcanic vents. To say that life was conceived in a warm womb seems intuitive: a reprise of our own fetal origins. But some scientists see a different beginning. They suspect that life began not in warmth, but in ice—at temperatures that few living things can survive today. The very laws of chemistry may have favored it, says Bada, who resides at the Scripps Oceanographic Institute in La Jolla, California. "We've been arguing for a long time," he says, "that cold conditions make much more sense, chemically, than warm conditions."

Miller's 25-year experiment, one of his last, provides a striking testament to the idea. "It's the kind of experiment that only Stanley could have done," says Antonio Lazcano, an origin of life researcher at the National Autonomous University of Mexico. "He's so obsessed with methodology. I like the idea of someone being obsessed with keeping the temperature down for 25 years."

Although life requires liquid water, small amounts of liquid can persist even 140 ºF below freezing. Microscopic pockets of water within the ice may have gathered simple molecules like the ones Miller synthesized, assembling them into longer and longer chains. No one can say where it happened—glacier, frozen pond, or iceberg. But the possibilities are breathtaking: a cubic yard of sea ice contains a billion liquid compartments—microscopic test tubes that could have created unique mixtures of RNA that eventually formed the first life.

If life on Earth arose from ice, then our chances of finding life elsewhere in the solar system may be better than we ever imagined.

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Miller's lab at the University of California in San Diego was full of glass vials—primordial chemical cocktails—aging like wine in a cellar. Some of these samples sat in freezers, others under the sink, and others in water baths maintained at various temperatures. They were part of an effort to understand chemical reactions that must have unfolded over thousands of years on early Earth. The location of every sample was stored in Miller's head; occasionally he'd hand one to a student to analyze.

When Miller handed his 25-year samples to a young graduate student named Matthew Levy, it was clearly something special. "I was scared," recalls Levy, who now does research at the University of Texas in Austin. "I was thinking, these samples are older than I am."

Levy burnt holes in his shirts over the next few weeks as he dissolved the samples with hydrochloric acid and ran them through an instrument, called an HPLC, to identify the chemicals that had formed. Red and green pens on the HPLC traced out tell-tale peaks on a scrolling strip of paper. Those peaks corresponded to a menagerie of molecules that early life would have found useful: eight different amino acids—building blocks which modern cells assemble into proteins; and eleven types of nucleobases—molecular building blocks of RNA and DNA.

“What was remarkable,” says Bada, “is that the yield in these frozen experiments was better, for some compounds, than it was with room-temperature experiments.”

Some people found it a little *too* remarkable.

When Bada and Miller submitted their findings to a top-tier journal called *Science*, they were rejected. A researcher who reviewed the manuscript for *Science* felt that those molecules must surely have formed while the samples were thawing—not while frozen at the ridiculous temperature of –108 ºF. So Miller, Bada, and Levy did more experiments to show that thawing played no role. They finally published their results in another journal, called *Icarus*, in 2000.

The skepticism they faced was understandable: everyone knows that chemical reactions slow down as the temperature drops. According to standard calculations, the reactions that assembled cyanide molecules into amino acids and nucleobases should have run 100,000 times more slowly at –108 ºF than at room temperature. Even if Miller ran his experiment for 250 years—let alone 25—he should have seen nothing.

This is the main argument against Miller’s experiment—and against a cold origin of life in general. But there’s more than meets the eye.

Strange things happen when you freeze things in ice. Some reactions slow down, but others, paradoxically, speed up—especially reactions that involve joining small molecules into larger ones. It’s caused by a process called eutectic freezing. As an ice crystal forms it stays pure: only molecules of water join onto the growing crystal, while impurities like salt or cyanide are excluded. These impurities become crowded in microscopic pockets of liquid within the ice. Crowding those molecules causes them to collide more often. Chemically speaking, it transforms a tepid 7^{th}-grade school dance into a raging molecular mosh pit.

“Usually as you cool things, the reaction rates go down,” concludes Leslie Orgel of the Salk Institute in La Jolla, California, who has studied the origin of life since the 1960s. “But with eutectic freezing, the concentrations go up so fast that they more than make up.” So reactions that require collisions sometimes speed up.

Freezing solves a problem that scientists have grappled with for decades. They believe that cyanide—created by ultraviolet light or electricity in the early atmosphere—was a key ingredient for building life’s molecules. But even if millions of tons of it rained down from the sky, it would never become concentrated enough in oceans or lakes to form those molecules.

Some people think that coastal lagoons on early Earth concentrated chemicals as the water evaporated—the same way salt flats form today. But that doesn’t work for cyanide, because it evaporates faster than water. “The strong point of freezing,” says Orgel, “is that you concentrate things very efficiently without evaporation.”

Freezing also helps preserve fragile molecules like nucleobases, extending their lifetime from days into centuries, and giving them time to accumulate and perhaps organize into something more interesting—like life.

Orgel and his coworkers first proposed these ideas in 1966 when he showed that frozen cyanide efficiently assembled into larger molecules. Alan Schwartz, a biochemist at University of Nijmegen in the Netherlands, took the idea further when he showed in 1982 that frozen cyanide could form a nucleobase called adenine. And Stanley Miller must surely have had the eutectic effect in mind when he laid down his now-famous samples in bubbling dry ice and acetone in 1972.

Their ideas follow a common genealogy. But ice has also lured others to study the origin of life: some of them arrived there by accident, and others pursued their obsessions to the ends of the Earth.

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In July 2002, a small skiff dropped Hauke Trinks on the beach of Nordaustland, a rocky island, smothered in glaciers and nearly devoid of plants: one of the furthest-north specks of land on the planet.

Trinks had come to Nordaustland to peer 4 billion years back in time—to an era when some astronomers think the sun was 30% dimmer, and the Earth may have been covered in ice.

As a chemist from the Technical University of Hamburg-Harburg in Germany, Trinks had become interested in sea ice 10 years before, while studying its tendency to accumulate pollutants from the atmosphere and concentrate them in liquid pockets. It occurred to him that a layer of ice covering early Earth’s oceans might also have assembled life’s molecules.

So with a few crates of supplies and two sled dogs, Trinks and his partner Maria Tieche hunkered down in a hut on barren Nordaustland for 13 months, studying the sea ice as it evolved through the seasons.

Each morning they rose from their cots, surveyed the temperature of the ice, and prepared the day’s experiments. During excursions on the ice, Trinks lay down and examined it through a microscope. To study the ice’s network of liquid pockets, he injected dyes into the ice and watched them spread.

Winter deepened, 24-hour darkness descended, and the mercury plummeted 30 degrees below zero. Trinks continued his experiments, chasing away polar bears by banging pans. One sunless day, he stumbled backward just in time as a walrus lunged upward through the ice and dragged several of his instruments into the ocean.

On land, Trinks built a makeshift laboratory table from planks of wood and discarded gasoline cans. With his hood pulled tight around his eyes, he examined slices of sea ice under the microscope. Turning a knob with a gloved hand, he nudged a metal electrode, one hundredth as wide as a red blood cell, closer to an ice crystal. The needle on his voltmeter jerked sideways, registering a sharp drop in voltage on the surface of the crystal. Trinks recorded his observations—always in pencil, since his ink pens had frozen.

By the time Trinks returned to Hamburg in 2003, he had formulated a theory that ice was doing much more than just concentrating chemicals. He imagined the ice surface as a checkerboard of positive and negative charges that grabbed individual nucleobases and stacked them together like Pringles in can, helping them to coalesce into a chain of RNA. “The surface layer between ice and liquid is very complicated,” says Trinks. “There is strong bonding between the surface of the ice and the liquid. Those bondings are important for producing long organic chains like RNA.”

Trinks met a chemist named Christof Biebricher at a scientific lecture in Hamburg. Biebricher was studying how the first RNA chains formed in the absence of enzymes that guide their formation in living cells. Trinks approached Biebricher with his sea ice theory. It wasn’t something that Biebricher naturally wanted to try—it sounded messy, impure—more like a margarita recipe than a legitimate scientific experiment. “Chemists,” says Biebricher, “do not like heterogeneous substances like ice.” But Trinks convinced him to try it in his laboratory at the Max Planck Institute in Göttingen, Germany.

Biebricher sealed small amounts of RNA nucleobases with artificial sea water into thumb-sized plastic tubes, and froze them down. After a year, he thawed the tubes and analyzed them for chains of RNA.

People had tried for decades to grow RNA chains under all sorts of conditions, without using enzymes; the longest anyone had gotten so far was about 40 nucleobases, which Orgel accomplished in 1982. But when Biebricher analyzed his own samples, he was shocked to see RNA molecules up to 400 bases long. In newer experiments, to be published this year, he’s even seen RNA molecules 700 bases long.

“That’s a new world record,” says David Deamer, a prebiotic chemist at the University of California in Santa Cruz. “Nobody’s made things that long before.” So fantastic are Biebricher’s results that some have privately wondered whether accidental contamination played a role. But Orgel defends the work. “It’s a remarkable result,” he says. “It’s so remarkable that everyone wants better evidence than they would for an unremarkable result. But I think it’s right.”

Biebricher wasn’t quite growing RNA chains from nothing. Before he froze his samples, he added an RNA template—a single-stranded chain of RNA that guides the formation of new strand of RNA. As that new RNA strand grows, it adheres to the template like one half of a zipper to the other. This phenomenon is important for life’s origin—it’s how the first genes, made of RNA, would have copied themselves. But it wasn’t the first step. Somehow the chicken and egg problem had to be solved: that first RNA molecule, which served as a template, had to appear.

Deamer and his former student Pierre-Alain Monnard (now at Los Alamos National Laboratory in New Mexico) have worked on this problem. They have run experiments frozen at 0 ºF for a month with no templates. They see RNA molecules up to 30 bases long—although most are shorter than 10 bases.

Those results are at least as good as others have seen in similar experiments without ice. But they raise a prickly question: how do you get from tiny snippets of RNA, to longer chains that could have acted as the first enzymes, doing fancy things like copying themselves or splicing pieces of RNA together to make longer pieces?

The shortest RNA enzymes known today are about 50 bases; most are over 100. To work effectively, an RNA enzyme must fold correctly, which requires having exactly the right sequence of bases. So how do you get from random 10-base fragments to this much larger, fine-tuned RNA machine?

A young scientist named Alexander Vlassov stumbled upon an answer. He was working at Somagenics, a biotech company in Santa Cruz, California, to develop RNA enzymes that latch onto the hepatitis C virus. But his RNA molecules were behaving strangely. Every time he cooled them below freezing to purify them, the chain of RNA spontaneously connected into a circle, like a snake biting its tail. As Vlassov worked to fix the technical glitch, he noticed that another RNA enzyme, called hairpin, also acted strangely. At room temperature, hairpin acts like scissors, snipping other RNA molecules into pieces. But when Vlassov froze it, it ran in reverse: it glued RNA chains end to end instead of clipping them.

Vlassov and his coworkers, Sergei Kazakov and Brian Johnston, realized that the ice’s dehydrated microenvironment was driving the enzyme into reverse. When an RNA chain is cut in two, a water molecule is consumed in the process, and when two RNA chains are joined, a water molecule is expelled. By removing most of the liquid water, the ice forced the enzyme to work in only one direction: joining RNA chains together.

They wondered whether an icy spot on early Earth could have driven a primitive enzyme to do the same. To investigate this, they performed reverse evolution experiments, where they introduced random mutations into the hairpin RNA, shortened it from its normal length of 58 bases, and even cut it into pieces—all in an effort to produce RNA enzymes that were as dodgy and imperfect as the first enzymes on early Earth surely would have been. These pseudo-primitive RNA enzymes did absolutely nothing at room temperature. But freeze them, and they became active, joining other RNA molecules together at a slow, but measurable rate.

It inspired a theory: that the first RNA enzymes—short, random chains like the ones they made in the lab—would have been inefficient, but ice could have acted as training wheels, allowing short segments of RNA to stick together and behave as a single, larger RNA molecule. "Freezing stabilizes the complexes formed from multiple pieces of RNA," concludes Kazakov. "So small pieces of RNA could be enzymes—not just large 50-base molecules."

While hairpin recognizes and joins only specific RNA molecules together, the pseudo-primitive RNA enzymes that Vlassov made in the lab were promiscuous; they grabbed and joined just about anything. Primitive enzymes on early Earth might have done the same, joining random segments of 5 or 10 RNA bases to create a wide variety of different RNA sequences. "So you have pools of millions of molecules," says Vlassov, "and under selective pressure you can select the first enzyme."

All of this would happen within microscopic pockets of liquid in the ice. "You have billions and billions of different possibilities," says Trinks, "because you have billions of these small channels"—each channel like a microscopic test tube, containing a unique RNA experiment. When the temperature rose in summer, the liquid pores expanded into a network of channels that mixed their contents. With winter, the liquid pores would contract and become isolated again, returning to their separate experiments.

With all of the mixing and matching, something special might eventually have appeared: an RNA molecule that made imperfect copies of itself. "You have something that is multiplying itself, and you have variation that is inherited," says Lazcano. "There, you have the onset of Darwinian evolution. I'm willing to call that living."

No one can really know how life began, but with the results of people like Miller, Biebricher, and Vlassov, the idea that ice played a role has gradually gained traction. "It is a plausible idea," says Orgel. "But of course it's just one theory amongst two or three."

Other ideas remain in the running—that mineral surfaces organized key molecules, or that volcanic sources synthesized amino acids. These theories needn't be mutually exclusive, either. Glaciers on early Earth could have scooped up mineral dust; volcanoes could have rained ash onto nearby sea ice. Primordial ice "must have been full of impurities," says Lazcano, "and those impurities must have had catalytic effects, enhancing the synthesis or destruction of some compounds."

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It's tempting to wonder what other frozen surprises Stanley Miller might have hidden away in his freezer—or perhaps in some bubbling thermos of liquid nitrogen. But we'll never know. Shortly after his 25-year experiment ended, Miller suffered a stroke. He hasn't returned to research.

In 2002 Miller's laboratory, with 40 years of samples, was finally emptied to make way for building renovation. Experiments that had run for years, perhaps even decades, were discarded. As Bada rescued a few items from his mentor's freezer, safety personnel stood by in hazmat suits—sent there by University officials concerned about rumors of toxic cyanide. Any sample that couldn't be identified was incinerated.

But it's not the end of the story. Events set in motion while Miller was active are still unfolding. It was a dinner conversation with Bada, in 1997, that originally prompted Miller to open his 25-year samples. Jupiter's moon Europa, with its water ocean covered by miles of ice, was garnering attention as a possible haven for life. Most people were excited about the ocean—but Bada wondered what sort of life-related reactions might happen in the ice. The results of Miller's experiment hardly seem discouraging.

Bada is now turning the techniques which he first used to study the origin of life toward finding life in other worlds.

Bada and Richard Mathies at the University of California in Berkeley have built an instrument that will fly on the European Space Agency's ExoMars mission, scheduled to launch in 2013. After ExoMars lands, it will drill 6 feet into the Martian crust—below the reach of solar radiation that would destroy delicate molecules. Bada's instrument will analyze the dust that is brought up for organic molecules—including markers of life, like amino acids. It will be the first experiment to look for life on another planet since the Viking Mars mission in 1976.

The instrument will actually distinguish between right-handed, "D" amino acids and left-handed, "L" amino acids. Life on earth uses only L amino acids. If the instrument finds an equal mixture of D and L amino acids on Mars, then it could mean that they weren't formed by living things. If the instrument finds mostly L amino acids, then it would be strong evidence for life on Mars. Yet it would leave open the possibility, as some people believe, that life on Mars came from the same source as life on Earth.

That would be exciting—but Bada hopes for a third scenario. “My great fantasy is that we go to Mars and find an excess of D amino acids,” he says. “That would be unambiguous proof of unique Martian life [unrelated to life from Earth]. I think we’d all just scream halleluiah.”