

Stanley Paul Leibo (1937-2014)

Stanley Paul Leibo studied the cryopreservation of embryos in the US in the twentieth century. Cryopreservation is a method of preserving biological material through freezing. Early in his career, Leibo collaborated with other scientists to study why cells were oftentimes injured during freezing. Later, Leibo and his team accomplished one of the first successful births using previously-frozen mammalian embryos. Leibo continued developing simpler and more reliable methods of cryopreservation and embryo transfer for many different species over the course of his career, such as the development of a one-step procedure of transferring fertilized embryos between cattle. Leibo's work to develop simple and reliable ways to cryopreserve cells and embryos enabled its use in a wider scope of research, including agriculture, reproductive medicine, and conservation.

Leibo was born in Pawtucket, Rhode Island, on 8 April 1937 to mother Fannie Leve who raised Leibo in the home of his grandparents, Sarah and Hyman Leve. Leibo's family migrated to the US from the Union of Soviet Socialist Republics in the early twentieth century before Leibo was born. Leibo attended high school at the Classical High School, an all-boys public school in Providence, Rhode Island. After graduating high school, Leibo began studying science at Brown University in Providence, Rhode Island, in 1954 and graduated in 1958. Then, in 1961, Leibo earned a master's degree in science at the University of Vermont in Burlington, Vermont, and in 1962, a master's degree in arts at Princeton University in Trenton, New Jersey. Around that time, Leibo married Bette Altman, who was working on earning her bachelor's degree in education at Lesley College in Cambridge, Massachusetts.

While working towards a doctoral degree at Princeton, Leibo also began working at Oak Ridge National Laboratory in Oak Ridge, Tennessee. There, Leibo worked with Peter Mazur, who at the time worked as a staff biologist researching cryobiology, or the study of biological material at low temperatures. He assisted Mazur in studying the effects of freezing on cells, beginning Leibo's career studying cryopreservation. Cryopreservation enables scientists to store or study cells through freezing. However, if precautions are not taken, freezing cells to temperatures as low as -196° Celsius can injure or kill the cells. Leibo and Mazur worked to understand what happens during freezing that causes harm to the cells, and how to protect against those factors that caused harm. Leibo completed his doctoral degree from Princeton University in 1963 as a result of his work on freezing single algae cells at Oak Ridge National Laboratory.

After obtaining his doctoral degree, Leibo joined Oak Ridge National Laboratory as a full-time employee to continue his research in cryobiology. In his first years at the laboratory, Leibo focused on researching the effects of freezing on bacteriophages, or viruses that infect bacteria. He joined as a staff biologist at the laboratory in 1965 and published his first paper on bacteriophage deaths during freezing in 1966 in collaboration with Mazur. The two collaborated throughout the 1960s to show that bacteriophage deaths, as well as the deaths of many other cells, during freezing were a result of two factors. The first factor they found to cause damage to a cell during cryopreservation involved changes in the salt concentration of the solutions within and outside of a cell as it froze. The second factor to cause injury to the cell during freezing involved the formation of ice within the cell during freezing. Mazur called that theory the two-factor hypothesis.

Leibo and Mazur used investigational methods in cryopreservation to draw their conclusions on the two-factor hypothesis. During cryopreservation, scientists suspend cells in test tubes containing solutions of water and dissolved salts. As temperatures lower during freezing, water crystallizes out of the solutions into ice, leaving higher salt concentrations behind. The changing salt concentrations push water in or out of the cell as part of the process of osmosis. For a cell to survive, scientists

must cool it rapidly enough to avoid long-term exposure to high salt concentrations in the solutions surrounding the cell. High salt concentrations can dehydrate the cell or reduce the stability of the cell's membrane, making it more likely to burst, as noted by James Lovelock, who researched cryopreservation in the 1950s in England. However, Leibo and Mazur detected that while a cell must be cooled rapidly enough to avoid injury by the first factor, it must also be cooled slowly enough to avoid ice formation within the cell, the second factor of freezing injury. Leibo and Mazur noted that a cell must be cooled slowly enough so water can move out of the cell before it crystallizes into ice. Ice formation can be lethal because ice takes up space within the cell, which can disrupt the cell's organization and functioning when thawed. For example, if ice were to form within a cell organelle in a eukaryotic cell, the organelle's shape would change as a result. When the cell is then thawed, the organelle will no longer have the shape it needs to function normally. Leibo and Mazur's work throughout the 1960s showed that ice formation within the cell was harmful to unicellular organisms, in their case, specifically bacteriophages.

In the 1970s, Leibo and Mazur began testing whether the two-factor hypothesis, which Mazur formed from studies of unicellular organisms, was also applicable to mammalian cells. Mammalian cells had fundamental differences from the unicellular organisms the pair had previously studied. For example, mammalian cells are structurally more complex and contain a variety of membrane-bound organelles. In addition, many but not all mammalian cells include a nucleus, which contains the cell's genetic material. Mammalian cells are more difficult to freeze than simpler bacteria cells because their membranes and the membranes of organelles within them are more sensitive to changing salt concentrations and ice formation than the solid cell walls often found in unicellular cells. Additionally, mammalian cells are more sensitive simply because they contain more kinds of organelles, which can become displaced or rendered ineffective due to ice formation. Leibo and Mazur sought to devise a reliable way to freeze mammalian cells that would ensure their viability once thawed.

In 1972, Mazur and Leibo successfully showed that the hypothesis was applicable to mammalian cells with nuclei in the paper, "A Two-Factor Hypothesis of Freezing Injury: Evidence from Chinese Hamster Tissue Culture Cells." That paper was the culmination of the years of research Mazur and Leibo had completed, and demonstrated the applicability of the two-factor hypothesis to both simple and complex cells. By showing that the risks of injury Mazur and Leibo had previously identified in simpler cells were also applicable to mammalian cells, the paper gave scientists the knowledge they needed to be able to preserve simple and complex cells with higher survival rates.

Also in 1972, Leibo and Mazur collaborated with David Whittingham, a scientist who studied embryos in England in the 1970s, to develop a reliable method for cryopreserving mouse embryos. Whittingham had published some successful methods for freezing and thawing viable mouse embryos the year before. Leibo and Mazur invited Whittingham to Oak Ridge National Laboratory to redo the experiment, but Whittingham could not replicate his initial success. The three then worked together to create a new way of cryopreserving the embryos that could reliably result in the birth of healthy offspring once thawed. That experiment ultimately resulted in one of the first documented successful births of a mammal using frozen embryos. A writer for The New York Times credited Leibo's study with laying the foundation for future work in mammalian embryo cryopreservation, including the practice of freezing human embryos in fertility clinics, a common procedure as of 2020. Following that study, Leibo's research began to focus more specifically on the cryopreservation of embryos rather than fundamentals of cryopreservation in general. Leibo continued studying the effect of freezing on mouse embryos and ova, or egg cells, into the early 1980s.

In 1981, Leibo left Oak Ridge National Laboratory to join Rio Vista International in San Antonio, Texas, as the vice president of research and development. Though Rio Vista included a fully equipped laboratory for research of embryo cryopreservation, it primarily operated as a cattle ranch and was not located proximally to any major academic research institution. Despite that, Leibo hosted monthly meetings at the ranch where any interested academics and professionals could discuss topics related to reproduction, development, or cryopreservation. He invited research scientists, zoo employees, medical students, veterinarians, and anyone else who was interested in the topic. Thomas Pool, Leibo's colleague who studied cryobiology in San Antonio, Texas, wrote that the meetings at Rio Vista were considered legendary by attendees, with discussions often continuing

long after sunset.

During his time at Rio Vista, Leibo worked to refine methods of cattle embryo cryopreservation. While working there, Leibo developed a one-step procedure for the thawing and transfer of cattle embryos. Previously, thawing frozen cattle embryos was a multi-step process that took up to ninety minutes. Scientists would have to follow meticulous protocols and place embryos in a series of different solutions containing different kinds of salts in order to maintain the balance of salt concentrations of the solutions outside and inside of the cells as the embryos thawed. Leibo developed a procedure that used only one kind of solution to safely thaw the cattle embryos. That reduced the time required to thaw the embryos to only ten minutes. The new procedure was simpler, more efficient, and cheaper than previous methods of transferring cattle embryos, while also showing similar success rates. That procedure helped make it easier to transfer frozen embryos of cows with desirable genetic traits, such as resistance to diseases, so that those traits could be introduced to new populations of cattle. It also aided in increasing the genetic diversity of other cattle populations and enabled ranchers to selectively breed cattle with the traits they desired. The New York Times credited that procedure with revolutionizing the cattle industry. Leibo patented his one-step procedure in 1983.

In 1988, Leibo took a job at Baylor College of Medicine in Houston, Texas, as a research associate professor. During his short time there, he continued studying the fundamentals of cryobiology. However, in 1991, Leibo joined the Animal Biotechnology Embryo Laboratory at the University of Guelph in Ontario, Canada, where he continued his research on cattle embryos, and later would become the director several years later. There, Leibo continued investigating the effects of freezing on the embryos of mice, cattle, and humans, and developed more efficient methods for embryo cryopreservation using rapid cooling.

In 1998, the University of New Orleans in New Orleans, Louisiana, appointed Leibo as the university's Doris Zemurray Stone Chair in Reproductive Biology and to the Audubon Institute Center for Research of Endangered Species. During his time working for the Audubon Institute throughout the 1990s and early 2000s, Leibo became involved with many projects dedicated to using cryopreservation for conservation purposes. Cryopreservation can be used to store cells and genetic material of endangered species, which scientists can later use to help threatened populations become more genetically diverse by transferring cells between populations, or also to fertilize female individuals when there are no males around. More genetically diverse populations are more adaptable to changing environments because they have a wider variety of traits that may be beneficial in different situations. Therefore, a more genetically diverse population is a healthier and more resilient population. Many of Leibo's projects focused on refining cryopreservation methods for the cells of new species of conservation interest.

Over the course of his career, Leibo published more than 100 scientific articles and twenty-three book chapters. He also was a member of various professional societies. From 1985 to 1987, Leibo served as the president of the Society for Cryobiology, a society made up of individuals and organizations dedicated to studying low temperature biology and medicine, and in 2005, was named a fellow of the society to recognize his contributions to the discipline of cryobiology. Leibo also was the president of the International Embryo Transfer Society, a society that focuses on embryo development, transfer, and cloning, from 1989 to 1990. The International Embryo Transfer Society awarded its Pioneer Award to Leibo in 2009 for his many accomplishments that advanced understanding of animal reproductive biology.

Additionally, Leibo worked on the editorial board of eight different journals, including the Society for Cryobiology's journal *Cryobiology*. Leibo's work developing reliable methods of cryopreserving mammalian embryos has been credited by Don Rieger, Leibo's colleague at the University of Guelph, and others as laying the foundations for the development of current in vitro fertilization technologies in humans that require the cryopreservation of human embryos. Rieger also asserts that the technology behind cryopreserving human embryos could not have developed without first understanding injury mechanisms for cells during freezing. Leibo's work to improve the success of cryopreservation of many different species has made it easier for researchers to maintain cell lines of species they are studying, preserve cell lines for conservation purposes, and use assisted reproduction for agricultural purposes or reproductive medicine in humans.

Leibo died of cancer on 25 March 2014 in Austin, Texas.

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