Mid-South
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Suspending Life At Oak Ridge

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Suspending Life

The temperature inside the test tubes held by Dr. Peter Mazur is minus 320 degrees – and the tubes contain life. Frozen in the tubes are mouse embryos, which Oak Ridge scientists will later thaw and implant in foster mothers.

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Suspending Life

Living proof of the success of their experiments are these mice resting in the hands of an Oak Ridge scientist. The mouse at left is the foster mother, with an offspring born normally from an implanted embryo which had been deep frozen for days.

SOME INTERESTING discoveries have been made lately in the field of cryobiology, the branch of science that deals with the effects of very cold temperatures on living things. One of these discoveries was made at Oak Ridge National Laboratory in East Tennessee, where a three-man team of scientists reported that mouse embryos had survived long periods of freezing at incredibly low temperatures.

After being frozen for eight days at temperatures going as low as 452 degrees below zero, the mouse embryos were thawed out and transplanted in "foster mothers," who then carried them to birth. When these thawed-out embryos developed into healthy, normal baby mice, the scientists uncorked a bottle of champagne and celebrated. It was a proud moment for cryobiology.

Soon after their findings were written up in the professional journals, two of the Oak Ridge scientists sat down to answer some questions about the possible implications of their work. They were in a small, upstairs laboratory in the Biology Building, the "Y-12" section of the Oak Ridge complex. Some secret stuff is still going on at Y-12, they say, but they don't say what.

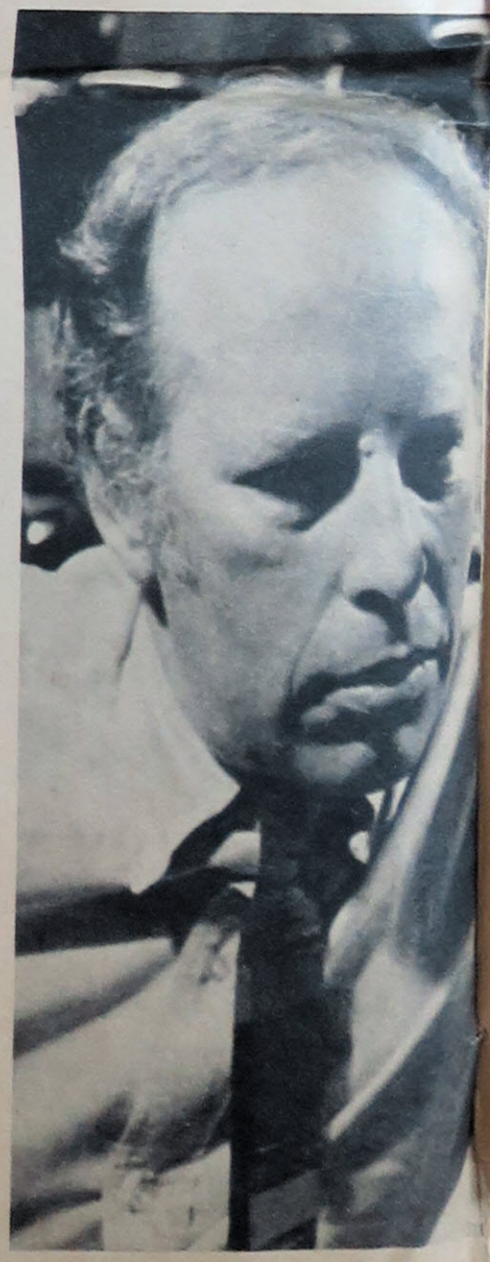
Dr. Peter A. Mazur is a calm, steady, thoughtful-looking man of 44. Dr. Stanley P. Leibo, who is 35, is quick, bright and humorous. Both have PhD's from Princeton.

The one who wasn't there was Dr. David G. Whittingham, an English scientist who had come over specifically to participate in the experiments. He went home as soon as the experiments were done.

DR. MAZUR spoke first about the science of cryobiology. "In some ways," he said, "it is a very old field. We have one reference that dates back to the 16th or 17th Century. However, modern cryobiology goes back only to about 1950. That was when it was discovered how to freeze human sperm."

Since then, Dr. Mazur said, the interest in cryobiology has been growing at a fantastic rate. "One of the main areas of concern has to do with the mechanisms by which cells are injured when frozen. If you think about it, the two most important things that determine the existence of living systems are proper temperature and the presence of liquid water. The temperature range where life is possible is very narrow. Basically, this is from the freezing point of water to about 150 degrees Fahrenheit. Almost 99 per cent of all organisms live between these two points. One way to study a scientific problem is to do something abnormal to the system and see how it responds. In our case, we freeze it and hope the response gives us some indication as to how a normal cell operates. This is the basic philosophy underlying our research."

Cryobiology also confronts some fascinating questions that have been around a long time. For example, says Dr. Mazur, "There are a number of organisms that routinely survive very low temperatures. Like plants. If you expose a plant to freezing temperatures in the summer, it dies. So the question is this: What mechanism does the plant use in order to survive these same temperatures in the winter?"



—Staff Photos by Charles Nichola

Oak Ridge scientists have succeeded in freezing mouse embryos, then later thawing them for normal birth in foster mothers.

By William Thomas

SINCE THERE was no chance of answering the question satisfactorily at this time, Dr. Mazur pressed on to the business of the mouse embryos.

"In the fall of 1971, Dr. Whittingham published a paper in a British journal in which he reported that he had been able to preserve mouse embryos for a brief period by freezing them. When we saw the paper, we were pretty excited about it. In a sense, this was one of the first instances where a whole mammalian organism had been frozen. One of the eventual payoffs might be the ability to freeze human organs. It is our belief that this is the only way organ transplants will ever come into widespread use. It's really a problem in logistics, you know.

"Suppose somebody has an accident. You could use the heart or kidney, if you had a recipient of exactly the same type ready within a few hours. But because of the complexity of matching up organs and patients, this is pretty unlikely. However, if you could freeze and catalog organs, you could do it. We thought the mouse organs might serve as simplified models for freezing more complicated systems."

Apparently, Dr. Whittingham began his experiments in England with something else in mind. He is with the physiological laboratory at Cambridge University, where a lot of work is done with strains of mice bred for special qualities. Maintaining the strain is both expensive and troublesome. Dr. Whittingham began looking for a better system after somebody pointed out how inefficient it was to be constantly mating and breeding these mutant strains of mice. It would be much better, somebody said, to put them away in the deep freeze and keep them there until they were needed. It was on this basis that Dr. Whittingham decided to see if he could come up with a way to preserve mouse embryos.

—Staff Photos by Charles Nicholas

The experiments were conducted by Dr. Peter A. Mazur (left) and Dr. Stanley P. Leibo, with the help of a colleague who has since returned to his British homeland.

THE SAME PROBLEM exists at Oak Ridge National Laboratory. There, hundreds of thousands of germ-free mice are kept in a building so spotlessly clean that a human being can walk in and contaminate it. These mice are being used to investigate the effects of low-level radiation and must be free of disease—so that if anything happens to them, the investigators will know exactly what caused it.

Dr. Whittingham left England and came to Oak Ridge last summer to help with the freezing experiments. "We invited him here to repeat, to extend and to analyze the work he had started," says Dr. Mazur. "He spent three very hectic months in our laboratory."

Dr. Leibo added, "One of the things we were hoping for was to find a way of preserving embryos on a long-range basis. Dr. Whittingham had not been successful in getting his embryos to survive after they had been frozen for more than 30 minutes. So, in terms of long-range preservation, he wasn't there yet. The problem was not solved. His stuff could not survive if frozen longer than 30 minutes."

Dr. Mazur nodded in agreement,

A summer freeze will kill a plant. How, then, does it survive winter?

then said: "We spent an uncomfortable three weeks here trying to duplicate Dr. Whittingham's experiments. We never were able to do it. We don't know why. All we know is that he had been able to freeze embryos at the eight-cell stage by suspending them in a protective solution called "PVP." (PVP stands for polyvinylpyrrolidone.) After we were unable to repeat his success, we decided to try a different suspending solution called "DMSO" (dimethyl sulfoxide.) That didn't solve all our problems, either."

WHAT FINALLY made the difference was the rate at which the embryos were frozen and the rate at which they were thawed out. Dr. Mazur explained it this way: "One of the major causes of freezing injury in the cells is the formation of ice crystals inside the cells. To avoid this, one has to freeze the cells more slowly. If it is done too fast, they freeze internally and that is lethal. We also slowed down the rate at which the cells were thawed out. Dr. Whittingham had frozen his embryos at about 60 degrees a minute. We cut that to about two degrees a minute. We also cut down on the thawing rate, and that made the difference. About 70 per cent of the cells survived."

Dr. Leibo described the feeling in the laboratory. "It was a very exciting time, because it got to the point where we couldn't do a bad

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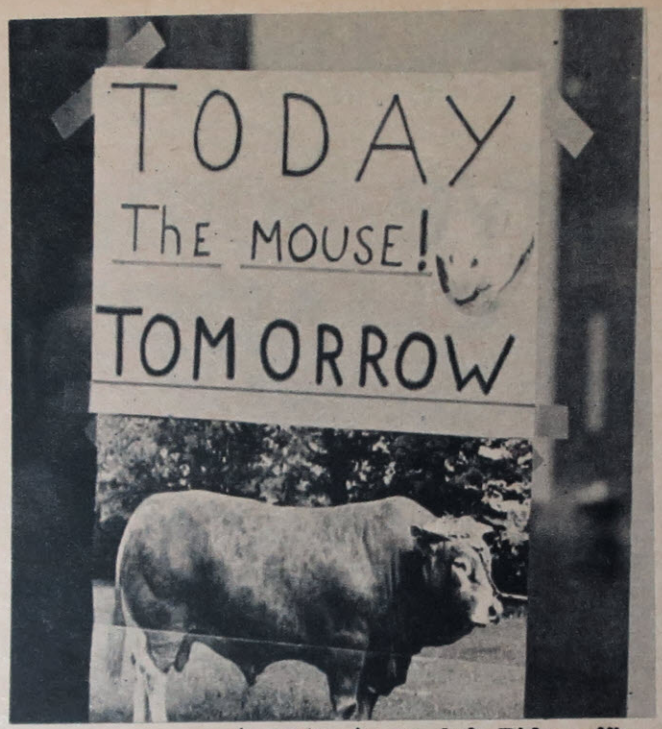

A poster in an Oak Ridge office shows one of the directions scientists expect their research to follow. Frozen embryos could revolutionize cattle breeding on a worldwide basis, they feel.

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The findings could revolutionize livestock production.

them and then implanting them in 20 or 25 separate cows, you could possibly produce that many calves. We could do the same thing in producing mutant strains of mice, which are very expensive."

Dr. Leibo said there have been cases already in which one kind of animal has been used to transport the embryo of another. "A paper has come out in England telling of embryos taken from a super-ovulated cow and put into a rabbit. Somebody tucked the rabbit under their arm, flew across the country and re-implanted the embryos in cows at the new location."

What the scientists have done at Oak Ridge is just as remarkable. Using liquid nitrogen and helium, they froze about 3,000 embryos and kept them at such low temperatures that all chemical activity stopped. In some cases, the temperature got down to within eight degrees of absolute zero (minus 452), the point at which there is complete absence of heat. Then, they thawed them out. Of 1,000 embryos that were placed in foster mothers, 65 per cent resulted in pregnancies and more than 40 per cent of these ended with the birth of normal mice.

"We could have frozen embryos for eight years instead of eight days and gotten the same results," Dr. Mazur says. "The only possible damage resulting from long-term storage would be from radiation that could accumulate over the years. However, we could protect against that by shielding — so it is not much of a problem."

HOWEVER, THIS does not mean that science is now on the verge of freezing human organs for organ banks. Nor does it mean that there is any more hope for people who would like to have their bodies frozen at death so they could be thawed out at some time in the future, when science has advanced to the point of biological immortality.

"The problem is one of sheer complexity," says Dr. Mazur. "We have been working with a relatively simple, eight-cell system. All along, we were right on the borderline of success. If we had been off just a little in any number of things—the cooling rate, the thawing rate, the suspending solution—we would have failed. We are just now beginning to find out why we were successful. We are also beginning to find out that we got lucky, or we couldn't have done it even on this simple a system in which all the cells are alike.

"OK, now consider the next step of complexity: the organ. It is composed of different types of cells. Yet, all of them must survive freezing and thawing or the organ itself probably cannot be preserved. The hard truth is that it just might not be possible to come up with any one freezing technique that would permit all of the cells to

survive. Different cells react differently to cooling rates, thawing rates, temperatures and solutions. It is a formidable problem. I don't think any of us can predict that it can be solved. However, the benefits are great enough that it is my personal feeling that somebody ought to try to work it out."

IF THE FREEZING of one organ presents such obstacles, then it is easy to see how the idea of freezing a whole human being becomes ridiculous in the eyes of the scientists.

"Here, you have hundreds of different types of cells to contend with," says Dr. Mazur. "Since these different types of cells would require different types of freezing to survive, it seems to me that the probability of being able to do it is so low that it's not worth talking about."

Dr. Leibo agrees. "One can't say it is impossible. But saying that doesn't mean that it is therefore possible. That's the flip argument the body-freezers are using. Since we won't say it's impossible, they take that to mean that it might be possible. That's not logic. It's not even good medical quackery."

> The idea of freezing a human being is deemed highly improbable.

Dr. Mazur goes even further: "If you took a cow and chopped it up into small pieces and froze them, you could make the argument that some day science might advance to the point where it could put the cow back together. Body freezing is at about the same level of probability. Personally, I wouldn't want to count on it."

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