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OAK RIDGE, TENNESSEE

AN INTERVIEW WITH DR. PETER MAZUR

FOR THE OAK RIDGE NATIONAL LABORATORY

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STOW: Today, we're talking with Dr. Peter Mazur. Peter came here in 1959 to join the Biology Division, and his career spanned almost four decades at the Laboratory. He's now at the University of Tennessee in Knoxville, and he has a very distinguished career to tell us about today. Peter, you are from New York City, I understand. You've come a long way from New York City to spend your life in the southeastern United States.

MAZUR: That's right.

STOW: What got you started in science?

MAZUR: I think, like many scientists I got interested really early, maybe in fifth or sixth grade. I'm not quite sure why, but for some reason, I got interested in biology, and that's where I've been ever since.

STOW: Was there any particular person in your life who may have sparked your interest?

MAZUR: Not really. My father was not scientific. He was in investment banking, so my interest was just a result of a mutation, I guess.

STOW: (laughs) Don't say that. You ended up going to Harvard for undergraduate and graduate work.

MAZUR: Right.

STOW: What were your career aspirations at that point? Did you envision working in a place like Oak Ridge National Lab?

MAZUR: No, I actually didn't. I assumed I was going to go to a university and, maybe with luck, end up working as a professor somewhere. And, it was after I'd finished my work as a postdoctoral researcher that I was introduced to Norman Anderson, who was at the Laboratory at the time. He expressed interest in having me come to ORNL. And, after I talked with him and later with Alexander Hollaender, I said, "Gee, it sounds great." They said I would be given the opportunity to just what I wanted to do, which at that time was cryobiology (the study of the effects of very low temperatures on living organisms). I took the position and worked at ORNL from 1959 until 1999 when I retired and joined the research faculty at UT.

STOW: You mentioned cryobiology, which is, of course, the freezing of biological materials. Was that a new technology in the 1950s?

MAZUR: Here’s the way it got started. I had a wonderful professor at Harvard, and he had a graduate student, who during World War II had collected hundreds of new species of fungi. Apparently, in the South Pacific, he noticed that the deterioration of cellulose products in his boxes was extremely rapid. He observed that the cellulose products dissolved overnight, and it turned out, the dissolution was due to fungal activity. So, he collected millions of new species and brought them back to Harvard. At that time, a freeze-drying technique called lyophilizing had just emerged for preserving biological substances. My professor once had a student who had looked at the viability of fungi and found it was very low. I came to him as a senior to do an honors project with him. He asked me, "Would you like to look at the viability of these biological samples?" I

said yes. And, it turned out that I observed that only a small percentage of the samples survived. So the professor said, "Well, maybe you'd be interested in trying to find out why they're being killed."

STOW: All right.

MAZUR: I said, "Well, if you're looking at freeze-drying, maybe you should look at freezing first." So I started freezing biological samples and I've been doing that ever since. So, that's how my interest in cryobiology started.

STOW: Was this an outgrowth of your dissertation research?

MAZUR: Yes. When I was in graduate school, my thesis was on freezing of fungi named *Aspergillus*.

STOW: So, you came to ORNL in 1959 and joined the Biology Division.

MAZUR: Correct.

STOW: Did you have much interaction with Alexander Hollaender at the time?

MAZUR: He was a very interesting person. I was interviewed by him and offered the position. He was a somewhat distant figure in some ways. You didn't get too chummy with him at first. But, of course, after you got to know him, you did. But, the opportunity in Oak Ridge looked great so I took it and worked with Norman Anderson for some time. Then I split off on my own as a group.

STOW: Well, that was pretty much the heyday of the Biology Division, wasn't it?

MAZUR: Well, it was.

STOW: Tell us a little bit about the work environment in the Biology Division.

MAZUR: Well, the emphasis was primarily associated with the effects of radiation on living organisms in one way or another. In 1953 the structure of DNA had just been elucidated by Jim Watson and Francis Crick ...

STOW: All right.

MAZUR: ... and so the predominant emphasis was on genetics and on tissues that might be adversely affected by radiation, particularly the bone marrow and other cells. One of the mysteries to me was, "Why did they ever hire me?" Cryobiology -- working on freezing of yeast and other organisms --didn't seem at all related to division missions. Well, it turned out later that Hollaender had thought there might be some advantage to preserving people's bone marrow by freezing it. If an individual was in a radiation accident, his preserved bone marrow could be transplanted to replace the marrow destroyed by radiation. Of course, I hardly ever did any work on that. However, we did some work with stem cells, which was interesting.

STOW: So, your work really related to the AEC mission because of Hollaender's foresight?

MAZUR: Well, I think so. I never knew that was the reason actually, but that apparently was the rationale for it, because I was just, you know, one person.

STOW: Did you work alone?

MAZUR: Well, at first. A technician and I worked together. Then later on, I had postdoctoral researchers. I guess the most famous one I had was a young fellow named Stanley Leibo, who had just earned his Ph.D. from Princeton. He was -- totally independently -- interested in freezing. So, he came and started off a fifteen-year association. I guess the culmination of it was the freezing of mouse embryos in 1972. But, it's always been a small group.

STOW: Okay. What are some of the underlying scientific principles in cryobiology? Is it something that we normally think of in our everyday working environment? Obviously, the freezing of organic materials and cells is going to do some damage there. So, tell us a little bit about what the limits are.

MAZUR: Well, I will, because my area of interest has really been the fundamental aspects of the field. Of course, most people, including me, are interested in the field's medical, agricultural, and genetic payoffs. But still, the fundamentals, I think, are important to achieving those aims. The first thing people often ask me is, "How long can living things be frozen?" And, the answer -- I think it's sort of interesting -- is that if you can get them down to liquid nitrogen temperatures, which is minus 196 degrees Celsius, calculations for some limited experiments indicate you can probably keep them 3,000 years or so. And, the reasons are that no biochemical or chemical reaction is going to occur at this temperature, and that the only source of damage really is background radiation, which can actually cause breakage in the DNA. But, background radiation is low enough so that it would take something like three to four thousand years to accumulate enough dosage to kill half a population of typical cells. So, storage is not the problem. The problems are: How do you get the cells down to these very low temperatures, and how do you thaw them out without irrevocably injuring them? It's a complex matter. Basically, as you freeze cells, ice forms outside of them first and then water tends to leave the cells. The water tends to remain unfrozen, flow out of the cells, and freeze externally. The cooling rate is a critical matter, because if you cool the cells too rapidly, their water is not able to leave rapidly enough. The water in the cells becomes increasingly super-cooled. Eventually, it'll freeze inside the cells, almost always a lethal event. So, the cells have to be frozen slowly enough so that the water can leave them completely. As a consequence, the cells dehydrate and shrink during the freezing.

STOW: I see.

MAZUR: That's one part. The other is that, even if you avoid this internal freezing, you usually have to add the so-called cryoprotective solutes present in common liquids, such as glycerol or ethylene. The beneficial effects of those solutes were really discovered in England around 1949, almost sixty years ago. So, the fundamentals of cryobiology are involved, to a large extent, with fundamental properties of the cells, such as the permeability of their membranes to water and the osmotic response of the cells to cryoprotective solutes -- that is, the change in the concentrations of external solutes and things of that sort. So, our understanding has to do more with these effects than the temperature effects per se.

STOW: (laughs) Well, it sounds interesting. I was concerned that the fluids would freeze inside the cell, expand, and cause breakage, just like something in your freezer.

MAZUR: It does, if they freeze. I'm not sure how much is due to the expansion, as it is just to damage from the growth of the crystals themselves.

STOW: Well, how do you go about assessing damage to a cell? I mean, do you observe the cells microscopically?

MAZUR: It depends on the cell. It depends on the cell type. For example, mouse embryos present an elegant system, because you can harvest them at any of the "pre-implantation" stages, which range from one cell to maybe 100 cells. They can be cultured in artificial media up from, say, the one-cell stage, to the so-called blastocyst stage, which has perhaps 128 cells. That's probably the best test. You freeze an eight-cell embryo, culture it, and then see if it develops into the blastocyst stage. For microorganisms, you look for cell division.

STOW: All right.

MAZUR: Then you determine whether they can form a colony. For tissue culture cells, you look for the same. For mouse sperm, you look for motility (the ability of sperm to move properly to an egg); whether the sperm wiggle or not is an indication of vitality -- or damage. Sometimes, certain vital stains can be used. Some living cells will tum a certain color but the dead cells will not. So, it depends on the cell type, but growth and cell division are probably the most definitive indicators of viability.

STOW: All right. So, you're in the Biology Division in, say, the1960s, investigating the fundamental science of cryobiology. What interest was taken by the Atomic Energy Commission headquarters in what you were doing?

MAZUR: Probably zero at the time. I really don't know the answer to that. Later on, I got my own funding from the AEC, so there was enough interest to provide limited funds.

STOW: Well, you were publishing papers in the open literature and you had a very prolific publication record.

MAZUR: I was working on yeast, but when Leibo joined me, we switched over to working on mammalian and tissue culture cells and, to some extent, bone marrow stem cells. On the basis of that work, we actually developed the "two-factor hypothesis" of freezing injury, which I alluded to earlier. That is to say, if you cool the cells too rapidly, you will kill them.

STOW: Yes.

MAZUR: If you cool them too slowly, you will kill them by too high a concentration of harmful solutes. We found that there was an optimum cooling rate for one type of cell. And, it was actually that work, I think, which primed us for the mouse embryo discovery that we published in *Science* magazine in 1972.

STOW: Yeah, and that was really one of the premiere contributions that you made.

MAZUR: It was an exciting period, actually.

STOW: Tell us a little bit more about your recollections of that period.

MAZUR: Well, what happened was that an investigator named David Whittingham had published a paper in *Nature* in 1971 in which he had purported that he was able to freeze early mouse embryos. Stanley Leibo and I got all excited about his work, so we convinced Alexander Hollaender to agree to pay for Whittingham's visit with us for three months.

STOW: Yes.

MAZUR: And, he came over in late May or early June. The first thing we did was try to repeat his results, which we were unable to do. And he even brought his own solutions. To this day, it's never been explained how he succeeded in 1971, because we were not able to repeat his experiment. But anyway, after about a week of frustrating work, Stanley and I were thinking about what might have gone wrong. We thought that he had cooled the embryos too rapidly. So, we thought through a protocol that we believed might work, and sure enough, in the first experiment we tried using slower cooling, we observed beautiful little eight-cell embryos. So we really solved the problem in that first week. The rest of the time we spent doing enough replicates to prove our approach worked. And actually by then, David Whittingham had become an expert in the rather difficult task of transferring these embryos into either the oviduct or the uterus of a recipient female and actually getting baby mice born live. A photograph of a surrogate mother mouse and her babies with a different fur color appeared on the cover of *Science* magazine in 1972, with our research paper.

STOW: You also had a 1973 article in *Science* magazine by Whittingham, Leibo, and you entitled "Maternal Influences on Mouse Embryos and Preservation of Mutant Strains by Freezing."

MAZUR: That was really a very minor paper. There was some belief that the physiology and the genetics of the mother might influence how the embryo developed. As I recall, this paper showed that was not the case.

STOW: The title of the '72 paper was "Survival of Mouse Embryos ..."

MAZUR: Yeah, it was the actual freezing one. And, it was a very exciting time for us personally. I think it was the start of what has turned out to be some pretty important applications and implications of embryo freezing, certainly.

STOW: Yeah, I want to come back to some of the applications and where the work is going in the future. But, speaking of publishing in *Science* and other journals, as I said a moment ago, you've got a very distinguished publication record here. And four of your publications have gotten a *Science* Citation Classics Award. What is that?

MAZUR: I'm not sure it exists anymore. *Current Contents* used to name authors or papers as "Citation Classics" if they were cited enough times by other researchers. I guess it was nice that four of my papers were named such.

STOW: Well, as long as we're on the topic, let me ask you more about authorship here. I happened to look through the list of your publications. And, early on in your career, you were the senior author on a lot of papers. Then later in your career, I notice you've not been the senior author.

MAZUR: That's true.

STOW: You intentionally share senior authorship with some of your coworkers. Why is that important?

MAZUR: It is important because as I've gotten older, I've tended to have more postdocs.

STOW: Yes.

MAZUR: And, postdocs do much of the creative work and nearly all of the lab work. The older we fogies get, the less time we spend on the bench and in the lab.

STOW: I understand.

MAZUR: So, it's appropriate, I think, that they become the senior authors and that I am not. So, that's the reason. I noticed that myself.

STOW: Okay. Well, as I looked through there, I saw that and I actually did the mathematics here on senior authorship. I saw a drastic fall-off in the last several years.

MAZUR: Yeah. In fact, there were a few of the early papers of which I was the only author.

STOW: You were.

MAZUR: And that was in my younger days.

STOW: Let's talk a little bit about the applications of cryobiology not only to our current activities and life cycle today, but also in the future. For instance, my daughter gave birth to a child about two years ago, and the doctor had the stem cells frozen. Is that part of what's grown out of the work you did?

MAZUR: Yes, it is. Maybe you'd like me to discuss the embryo business first or the implications of it first.

STOW: All right, let's do that.

MAZUR: I think, partly, or mostly, as a result of genetics, there's been an enormous interest in creating so-called "transgenic strains" of mice, which are mice that have received purposeful mutations as a way of modeling human diseases. And, that's certainly a major emphasis at the ORNL Mouse House. But it's created a problem in that the maintenance of living colonies of mice is an expensive proposition, and the number of mutant lines is growing at an astronomical rate. Other ways of preserving the germ plasm of different mouse strains are needed.

STOW: Okay.

MAZUR: So when Stanley Leibo, David Whittingham, and I were able to freeze mouse embryos, it immediately became evident that this procedure was going to be an important way to preserve genetic lines. I think Liane and Bill Russell took it up, to some extent, in the early days, and started preserving some of their mutant lines. But, the biggest player in the field became the Jackson Labs in Bar Harbor, Maine. I have forgotten how many mouse strains and how many embryos they've frozen, but it was in the millions of embryos. Once you freeze them, they're not going to change for a millennium.

STOW: Yes.

MAZUR: So, freezing provides a cost-effective way of preserving the less frequently used ones. It also provides insurance. Jackson Lab, for example, had some major fires that destroyed many of their lines. They were, fortunately, able to resuscitate them, or re-derive them from strains at some other labs, but if they had a frozen stock, it would have been much easier.

STOW: Okay.

MAZUR: So, there's been this enormous, important push for mice, but our freezing technique has had implications in many other mammalian species as well -- for example, the cattle industry. Well, independent of anything we did, for years the cattle industry has frozen bull sperm, which has been the major source of sperm in the dairy industry. I think ninety percent of all dairy cattle are conceived using frozen sperm. And actually, that goes back to this 1949 work that I mentioned from England, where they discovered the protective effect of glycerol. Using glycerol, the cattle industry routinely freezes sperm. With our discovery of how to freeze mouse embryos without damaging them, there was immediate interest in trying to freeze cattle embryos. Stanley Leibo and I worked on freezing cattle embryos with UT researchers at CARL -- the Comparative Animal Research Laboratory in Oak Ridge.

STOW: That's right.

MAZUR: We tried it ourselves but were not able to succeed with cattle in Oak Ridge, as other groups quickly did. So, a smaller industry has grown up now, preserving the female side of cattle -- that is, embryos from a prize cow. Generally associated with embryo freezing is a technique called "super-ovulation" in which hormones are injected. It's used with humans, too. Instead of one or two eggs being shed, fifteen or twenty eggs are produced.

STOW: All right.

MAZUR: So, that's potentially a way of expanding the output of a prize cow, just as frozen sperm can expand the output of a prize bull. So, that's created a sizable sub-industry, shall we say. Embryos from some twenty-six species of mammals have been successfully frozen. There are a few species that have not been frozen, like the pig, but most of them have. And, one of the applications of our technique is to preserve the germ plasm of endangered species. And the other possible application is to freeze embryos of non-mammalian systems.

STOW: Yeah, you've worked on insects.

MAZUR: I've worked on mosquitoes and fruit flies. There's also a lot of interest in trying to freeze the embryos of fish, again for genetic reasons, primarily. And, I have a colleague who is trying to freeze the eggs or the embryos of the Zebra fish, which is sort of a "mouse model equivalent" of non-mammalian forms.

STOW: All right.

MAZUR: In fact, a woman from Germany won a Nobel Prize recently for working with Zebra fish. It's paradoxical though, that cryopreservation of mammalian embryos has, once we found out how to do it, ended up being a relatively easy thing to do, whereas cryopreservation of non-mammalian systems has ended up being a very difficult thing to do. In fact, the eggs of the fruit fly *Drosophila* and the house fly are the only ones I know of that have been successfully frozen. There are some reasons for why it's so complicated.

STOW: I was going to ask, and, if you can give me a simple answer, great. Let's go back to the mammals.

MAZUR: Sure.

STOW: You said that twenty-six different species have been frozen. But pigs, we've not been able to freeze. What makes a pig different from twenty-six other species?

MAZUR: Good question. I'm not sure. One thing is that most cells and embryos are not particularly sensitive to temperature change itself, aside from ice formation, but the pig embryo is very sensitive to temperature change, even above freezing temperatures. So, that's one reason. The other may be that the pig eggs contain a large quantity of lipids, and some correlation seems to exist between lipid content of these eggs and embryos and the difficulty in freezing them. But what that connection is, I don't think anybody knows. Leibo and a Japanese investigator did some innovative experiments in which they centrifuged the eggs, collected all the lipids at the bottom, and then sucked the lipid out of most of the eggs. They found that when they removed those lipids, they reduced both the chilling sensitivity and the freezing sensitivity to some extent. So, there is evidence for that. But the answer is vague and I don't really know the answer.

STOW: So, cryobiology is not an old field or sub-discipline of biology, by any means.

MAZUR: No, it isn't.

STOW: And, there's apparently a lot of work yet to be done.

MAZUR: Yeah, especially when you get into the more complex systems.

STOW: Where is that work being done now? Are we continuing some of that work here at ORNL?

MAZUR: There's no cryobiology research being done at ORNL anymore. Dabney Johnson and some of the other people at the Mouse House are making use of the ORNL technique for freezing sperm and embryos, again in relation to moving mice over to the new Mouse House at ORNL from the old Mouse House in an ORNL Biology Division building at the Y-12 Plant.

STOW: I was going to ask how this would help on the move.

MAZUR: Many of the mutant lines in the Mouse House over the years gradually became "infected" with various endemic microorganisms, which made them, in a sense, unacceptable to outside labs. So, when the decision was made and approved to build the new facility at X-10, the decision was also made to provide a clean, germ-free source. Well, there's no way to clean up the mice from the Y-12 area. There were two ways to do it, basically. One is that you can take home pups, viruses, and bacteria. And the other way is to re-derive clean mice from frozen embryos or frozen sperm.

STOW: Once they get over to X-10.

MAZUR: I think the decision made was that the actual physical mice were not going to be moved. Instead they will take frozen mouse embryos, thaw them in liquid nitrogen, and then transfer them into germ-free recipient mice in the new facilities. Our embryo freezing technique has been of major importance to this new facility.

STOW: You know, as you describe the processes of freezing and everything associated with it, I keep thinking about patents. Is there anything that is patentable here?

MAZUR: Yeah ...

STOW: Did you ever get a patent or anything?

MAZUR: No, we never did. I suppose that was a dumb thing not to have done. But no, I've never had a patent. I mean, yes, there are a number of aspects ... I don't know who has patents on what, but there are certain aspects that are patentable. But I really don't know any more details on it than that. I guess it's because of my somewhat academic background that I think these things ought to be in the public domain, and mine are.

STOW: Well, I keep coming back in my mind to the relevancy to the AEC and DOE mission, and I think I understand that a little bit better now. Yet, you've had a number of other sponsors over the years. The National Institutes of Health and even the State of Tennessee helped support some research you did. Can you expand on other work that you've done?

MAZUR: Sure. Many people at ORNL get all their funding from DOE. Some get only partial support. It was up to the principal investigator to find other sources of support. That has really been my case for ten or fifteen years. DOE supported about half of my research, and the other half I'd really have to get myself, especially if I wanted postdocs and new equipment. So, my main sources of biology funding in the United States have been the National Science Foundation and the National Institutes of Health. The NSF funded all our *Drosophila* (fruit fly) work. It came about because the *Drosophila* community of researchers has major funding support from the National Science Foundation. The researchers became increasingly concerned with the maintenance of thousands and thousands of mutant lines of *Drosophila.*

STOW: All right.

MAZUR: And so they persuaded the NSF to put up funding for two groups to study this problem. There was my group and the group at Cornell University, headed by Peter Steponkus. Both were successful. After the *Drosophila* work, a laboratory director at NIH named Lou Miller, who was heavily involved in malaria research, contacted me. Malaria is really a terrible disease. It infects something like 500 million people a year and kills about two million a year, mostly in Africa and mostly young children. It's been very refractory to solution. One proposed solution is to genetically modify the mosquito, so that it becomes incapable of carrying the parasite that causes malaria, the plasmodium parasite. Lou Miller was involved in that.

STOW: I see.

MAZUR: But, as is the case with the mice, creating all these potential candidate transgenic lines presents a problem. It becomes very difficult to maintain them in the living colonies, especially mosquitoes, which have to be blood-fed. He and I started a collaborative attempt, and then I got NIH support for that work. Unfortunately, it hasn't been successful because of complications related to the properties of the mosquito eggs. So far, we haven't been able to find a solution but somebody will sometime. We also had support not only from NIH but also from the U.S. Department of Agriculture for freezing mouse sperm. This is another way of preserving germ plasm, other than just the embryos. It's sort of strange, really, that one of the first cell types frozen in 1949 was cattle sperm.

STOW: Okay, yes.

MAZUR: Attempts to freeze mouse sperm were totally unsuccessful until 1990. And even in the ensuing decade, it's been very difficult to do so repeatedly. So, I have been working with a colleague, then at Indiana, now Missouri, to try again to understand the fundamental problems involved with freezing mouse sperm. I think we have succeeded in developing a pretty reliable freezing technique for mouse sperm.

STOW: You said 1990?

MAZUR: 1990. The first report of freezing mouse sperm was 1990.

STOW: I'll be darned.

MAZUR: Even though the first report of successful freezing of mammalian sperm was 1949. Again, it's complicated as to the reasons why. Basically, the mouse sperm have a set of peculiar sensitivities that makes it difficult.

STOW: You mentioned that NSF has supported some of your work. We operate, of course, with the philosophy that NSF doesn't support work at the national labs. How'd you get around that?

MAZUR: I think we got NSF funding because Alexander Hollaender (first director of the ORNL Biology Division) had set up this collaboration with the University of Tennessee. The NSF supports lots of research at universities.

STOW: Yes.

MAZUR: In effect, the Biology Division had a UT-ORNL Graduate School of Biomedical Sciences that was physically housed here at ORNL, and we were able to route grants through UT in that way.

STOW: The money officially went to UT then.

MAZUR: Yes, that's right. Somebody skillful worked that out.

STOW: That's true. You came here in 1959. That was during both the heyday of the Biology Division and Alvin Weinberg's early tenure as Laboratory director.

MAZUR: Right.

STOW: Did you get to know Weinberg?

MAZUR: I did, indeed.

STOW: Tell us a little bit about your interactions with him. Weinberg had a reputation of attending annual information meetings, sitting in the front row, and asking penetrating questions of young investigators. Were you ever his prey?

MAZUR: That's an interesting point. Yeah, I was as a matter of fact. I guess it was the first or second year I was in the Biology Division. I was tapped to make one of these presentations. And, you're right. There he was sitting in the front row. So, I went through my spiel about fundamental stuff, and he looked at me and said, "That's very interesting, but what's the use of it all?" So, that took me a bit aback.

STOW: Were you able to give an acceptable answer?

MAZUR: I guess so. I wasn't fired. We became very good friends. I have a tremendous admiration for him.

STOW: Oh, yes.

MAZUR: In fact, he started out as a mathematical biologist.

STOW: Well, as a biophysicist ...

MAZUR: A biophysicist. He got his Ph.D. degree under a mathematical biologist at the University of Chicago named Raschevsky ...

STOW: All right.

MAZUR: But, he's always had, not only an interest, but, I think, a deep understanding of biology. He was a believer in big science and big projects. But he understood that biology is often not that way. I mean, some aspects of biology are, like many of the genome analyses. But, a lot of biology

is still done by small groups, and appropriately so, I think. And, he realized that. So, he was, I think, a powerful supporter of biology.

STOW: Very much so, and a powerful supporter of virtually all aspects of the Laboratory.

MAZUR: Yes.

STOW: Today, the life sciences, and, perhaps more specifically, biology, gets a little bit of a bad rap when it comes to scientific misconduct, fabrication and falsification of data, and so on. There are reasons for that because it's easier to do with biological systems than with physical systems, for instance. Was that ever a concern to you during your career here -- that the life sciences would be a discipline where scientific misconduct might arise?

MAZUR: I don't think the thought ever passed through my head. I don't know if it's a different thing. There are aspects about cryobiology that raise ethical questions, but they're a different kind. The chief ethical question, of course, concerns (the creation and cloning of) human embryos. Freezing human embryos can be done, but we didn't go into that.

STOW: Well, I want to talk to you here shortly about the future ...

MAZUR: Of the twenty-six different mammalian embryos that have been frozen, one includes the human embryo. There are lots of ethical questions that have been raised about freezing human embryos. There have been cases, even here in Knoxville a few years ago, as to who has custody of frozen embryos and what you should do with them if you collect twelve human embryos and use three of them. What happens to the other nine?

STOW: I remember that, yes.

MAZUR: Who owns these embryos, and who are their parents, for examples? But, on the other question about scientific misconduct, no. It really didn't enter my mind, I don't think.

STOW: All right.

MAZUR: And, I haven't personally been involved in any such matters.

STOW: Let’s talk a little bit about looking toward the future. And, then I want you to look back on your career also. But, what do you think the future of cryobiology is? And, what are the big challenges that the discipline is facing right now?

MAZUR: We've been talking mostly about embryo freezing, but there are other areas, I think, which are really significant and have considerable potential. For example, a whole slew of medically important cells and tissues can be preserved by freezing, an important advantage.

STOW: Yes.

MAZUR: One of the important ones is human red blood cells. The successful freezing of human blood cells goes back a long way. But, there are a number of blood centers now that freeze blood.

Freezing is particularly used with the rare blood types. It's an expensive proposition. So, it isn't used routinely, but there are rare blood types, other than the usual A, B, and O types and Rh ones that sometimes need to be matched. So, they can be successfully frozen. Some types of white blood cells with important uses can be successfully frozen. Even more interesting types are stem cells, which have received an enormous amount of publicity recently. The public has heard that stem cells are the primitive progenitor cells, that, presumably, under proper conditions turn into specialized cells.

STOW: Yes.

MAZUR: Stroking can be induced to develop stem cells into mature tissues. And, of course, it's come up with the cloning issue. One of the arguments against using cloning for therapeutic purposes is that stem cells can do everything a clone can do. Another big use for stem cells is in actual treatment of diseases. For example, for somebody who has leukemia, one treatment is essentially to remove all of the cancerous bone marrow cells or sort of lethally irradiate them and then, transfuse normal stem cells back into the bone marrow to repopulate it. Of course, the cells have to be the right type, so it would be an enormous advantage if you could freeze the stem cells of the right type, or even from the same individual, and re-transfuse them. Fortunately, the freezing of stem cells is fairly easy to do. I think you commented earlier, about umbilical stem cells.

STOW: Yes.

MAZUR: The umbilical cord is a rich source of stem cells. There's been a lot of discussion about that, and, in fact, there are even groups actually freezing the stem cells from a newborn baby. They preserve the baby's umbilical stem cells in liquid nitrogen as insurance in case they might be needed sometime in the future. And, there's no question -- it's technically feasible. Also, the stem cells can be preserved for hundreds of years, if necessary. Now, there may be economic questions about this approach, but not technical questions.

STOW: I understand. Did that work on freezing stem cells and other things that you mentioned grow, either directly or indirectly, from the work that you've done here at the Laboratory?

MAZUR: Well, I think we certainly had an impact. I like to think that understanding the fundamental aspects of the field helped others. Otherwise, if you try to do it all empirically, you have hundreds of different cell types and you're trying to develop hundreds of different recipes, so to speak. And, if you understand the fundamentals, as I think I illustrated with the embryos, it can lead to solutions much more quickly and readily.

STOW: Well, let me ask you to look back over your career ...

MAZUR: But, before we do that, you'd asked about one other aspect about the future of organs. We should just say a few words about that. One of the target desires is to be able to freeze whole organs, such as hearts, kidneys, livers, and lungs. If such a capacity existed now, it might have reduced the chances of the tragedy at Duke University a few days ago, where an organ of the wrong blood type was implanted. Freezing of organs has been a formidable problem, but there are strong indications it can be done. We mentioned freezing of *Drosophila* eggs. Well, a *Drosophila* egg actually has 50,000 cells, so that's a complex system that can be successfully frozen. We ourselves, and others, have done work on freezing pancreases to preserve the insulin-producing

function of the islets of Langerhans. The cells of most organs can be easily frozen. That is to say, if you can take the cells from a heart and dissociate them, they can be frozen easily. The same thing is pretty much so of kidney cells and liver cells. But, when you try to freeze a whole organ, the problems become much more formidable, partly because of the size. Partly because an organ is more than just a collection of cells, interactions occur among the cells, and the overall architecture is important in the organ's function. And so, the progress in freezing organs and whole tissues has been much slower, but it's going to come sooner or later. For example, corneas can be frozen quite successfully now. But, when organ cryopreservation comes, there may be options of setting up organ banks with tissue-typed organs ready for transplant. So if some recipient needs a new liver or a new heart, you won't have this mad scramble of trying to find a suitable donor again, as was exemplified just this past week. And so, I think that has enormous potential, and I would like to see more funding put into it.

STOW: Do you think we're, what, five, ten, twenty years away from whole organ freezing?

MAZUR: Oh, it's probably going to be different for different organs.

STOW: Let me ask you to look back on your career. And, can you identify any single achievement that you're most proud of and that you really want to speak about?

MAZUR: Well, I think I'm most proud of a paper I published in 1963, which just seems like yesterday to me ...

STOW: Let me find that ...

MAZUR: It came out in the *Journal of General Physiology,* and it was the paper that set up the modeling for the shrinkage of cells during freezing and the mathematical equations that described it and really set up, I think, the major part of the fundamentals of the field. It's a pretty arcane paper ...

STOW: Is that the kinetics of water loss, and so on?

MAZUR: Yes, that's the one, on water loss ...

STOW: All right.

MAZUR: I think that's the one I'm proudest of. It probably has had the most impact on the field, or on the fundamentals of the field. And, I guess, the second one has to be the mouse embryo freezing paper in 1972 because that opened up what I think are a lot of important implications of cryobiology for various disciplines.

STOW: Is there any particular accomplishment that you wish you had done, either in theory, or that you tried and failed at?

MAZUR: Well, the mosquito problem is one.

STOW: Yes.

MAZUR: So far, I'd say, we have failed at freezing one type of white cells– the so-called granulocytes, which are also involved in disease fighting. We made an attempt to freeze them, but that failed. We're not alone. A lot of people made futile attempts to freeze them. But, I think the mosquito freezing challenge is a more significant failure, if you want to put it that way. But it's not a total failure in that we've not done something ...

STOW: Just not gotten there yet ...

MAZUR: ... not gotten there, but I think some important principles have been elucidated because of our work.

STOW: You've received numerous awards and honors over the years. You've got an R&D 100 Award, you're an ORNL corporate fellow and chairman of the ORNL Corporate Fellows Council, and you are listed in *Who's Who in America* and *Who's Who in the World*. We could list a tremendous number of such accolades. Is there any particular award or honor that you're most proud of?

MAZUR: Hmm -- good question. None is coming to mind.

STOW: All right.

MAZUR: Although, I must say, although it wasn't an award, I was honored by Alvin Weinberg's nomination of me for the Japan Prize, which I didn't get. It was interesting that the one who did receive it was Chris Polge, who discovered the freezing of sperm in 1949. But, I was pleased that Alvin had thought highly enough to nominate me. And, Al Trivelpiece put me up for an award I didn't win -- I forgot whether it was a technology medal or something else.

STOW: Speaking of Trivelpiece, Weinberg, and others -- you've rubbed elbows with a lot of fairly famous scientists, engineers, and administrators over the years. Do you feel that any of them stands out head and shoulders above the others or has had an influence on your career?

MAZUR: Well, Alex Hollaender certainly had an influence, because he was a remarkable guy in the sense that he realized that his reputation really depended on the accomplishments of the scientific staff that he assembled. And so, I think many people were given their head. Basically, you can either do well, or hang yourself, so to speak. I mean, if you were not productive scientifically, you might be out. So, he was a tremendous influence. Alvin Weinberg, while not so much in my field directly, was a mentor for me in science. The one interesting aspect of my career that we didn't touch on at all was the time I spent on the Space Science Board of the National Academy of Sciences, which gave advice to NASA. And, I was involved with the Viking Project, which had the only lander that landed on Mars. One of its purposes was to look for signs of life. There are lots of questions about Mars that are relevant to cryobiology because this planet is perpetually locked in very low temperatures. As you know, important questions are being asked concerning whether life still exists on Mars, which is, I think, unlikely, or whether it might have once existed, which is more likely.

STOW: If you were a betting man, would you say life did exist on Mars?

MAZUR: I think so, because there's strong evidence that liquid water was probably present. And, liquid water is really, in some ways, the essence of life, and actually, the central factor in all of cryobiology.

STOW: Well, true. Now, it is interesting you would wind up our interview by talking about Mars, because, as we started out with you telling a little bit about the history of cryobiology, I began to think about science fiction stories and the freezing of monsters and thawing them out. As we wind up this interview, we're back on the science fiction aspect of the planets and freezing of living things. I tend to agree with you. I think probably life did exist on Mars, and it may be there today. I don't know.

MAZUR: It's too bad in a sense. If you froze a few living organisms now in liquid nitrogen, they would probably still be alive in 10,000 years. After scientists thawed out the organisms, they could actually have a direct lab bench comparison with current organisms of the same species, enabling them to demonstrate that evolution has occurred.

STOW: Yes.

MAZUR: Because 10,000 years is probably long enough to see signs of evolution.

STOW: Sure.

MAZUR: So, you could compare something that lived 10,000 years ago with something of the same species that is alive today, side by side on the same table or desk.

STOW: Interesting.

MAZUR: That would be sort of fun.

STOW: Maybe we ought to go and make a movie together or something like that.

MAZUR: That's right.

STOW: Ok, thanks very much, Peter. It's been a good interview, and we've enjoyed it.

MAZUR: Well, I've enjoyed it too, very much. Thanks.

----------END OF INTERVIEW ----------