The icy world of organ freezing

Shortcuts to organ freezing have not been successful. Cryobiologists are now probing the basic mechanisms.

by Joan Arehart-Treichel

Scientists have been curious about the effects of cold on life for several centuries. But serious scientific investigations into the biological effects of cold are essentially a 20th-century phenomenon. Efforts to freeze and revive organs, in fact, are quite recent, and have been spurred on by organ transplants of the past decade. If whole organs could be frozen and stored, particularly ones immunologically suitable for recipients, surgeons would be able to perform far more transplants and have better prospects of success.

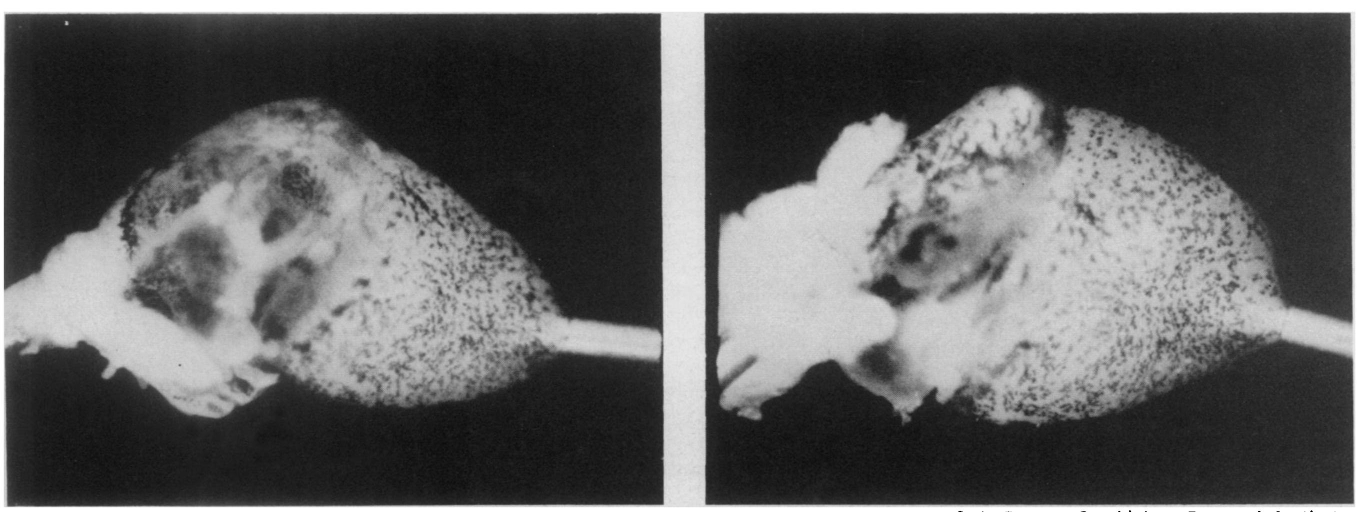
For all their efforts, though, cryobiologists (biologists who study the effects of cold on life) have not been very successful with organ freezing. In the area of heart freezing, for example, G. L. Rapatz of the Cryobiology Research Institute at Madison, Wis., has managed to freeze and revive a frog heart. But the frog heart is considerably less complex than the mammalian heart, where nutrients and oxygen are transported through coronary blood vessels. Some mammalian embryonic hearts have been frozen and revived. A number of mammalian cardiac muscle fibers have been frozen and reactivated. Some investigators have managed to cool adult mammalian hearts to minus 20 degrees C. (minus 196 degrees or lower, would be the ideal storage temperature) and revive them. But in nearly all of these instances the hearts were not kept cold for more than 20 minutes. One exception, is when F. G. J. Offerijns of the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service cooled them for six hours. In any event, nobody to date has cooled whole mammalian hearts any lower or longer and revived them. Organs would have to survive freezing temperatures if they were stored for any length of time.

Harold Meryman of the American National Red Cross Blood Research Laboratory in Bethesda, Md., and a cryobiologist who has probably been in the red-blood-freezing business as long as anybody, sheds some light on why mammalian organ freezing hasn't been more successful. A quarter century ago, biologists accidentally discovered that the chemical glycerol can safeguard bull sperm from the harmful effects of freezing. Then optimal freezing and thawing rates for the sperm were found. Glycerol was subsequently found to also protect human red blood cells and human sperm, and optimal freezing and thawing rates for these cells were also determined. It was possible to set up banks for the long-term preservation of animal and human sperm and of human red blood cells. The stage appeared to be set for tissue and organ freezing.

"The pot of gold appeared to be just around the corner," Meryman recalls. "It was a kind of race. We were all guilty. I certainly was." But cryobiologists were fooled. Freezing and reviving mammalian tissues and organs have not panned out by sheer trial and error experiments—trying to find the right cryoprotective agents and the right freezing and thawing temperatures. Consequently some cryobiologists decided the only way they will ever be able to freeze human organs for long periods and revive them is to first understand the effects of freezing and thawing on various cells and tissues in various organs.

Although probing the basics is, understandably, tedious, cryobiologists are making progress. Some of their latest efforts and results were reported in Washington at the recent annual meeting of the Society for Cryobiology. Particularly interesting is work reported by Rapatz and Offerijns, not just because they have good track records in organ freezing, but because they are focusing on one of the most vital organs—the heart.

During the past two or three years, both cryobiologists have been largely dealing with "pacemaker" cells of the heart—those cells that beat and contract even when removed from the heart. Rapatz has managed to cool these cells to minus 80 degrees C. and revive them. Offerijns has actually frozen them, using a cryoprotectant agent called dimethyl sulfoxide (DMSO), and revived 80 percent of them successfully. But both scientists have noted



G. L. Rapatz, Cryobiology Research Institute

Frog heart before and after freezing. Freezing and reviving mammalian hearts have not yet been accomplished.

that the membranes of these pacemaker cells appear to be particularly vulnerable to freezing and thawing. This is not an unexpected finding, since other recent evidence suggests that cell membranes suffer from freezing (SN: 7/22/72, p. 54).

Offerijns believes, though, that the membranes are particularly vulnerable because they experience upsets in the movement of ions during freezing. This movement might be precipitated directly by the low temperatures, or perhaps by osmotic pressure exerted on the membranes by cryoprotectant agents. In one set of experiments the Amsterdam biologist found that although movement of ions across cell membranes continued after freezing, the cells no longer contracted. Normally contraction follows ion movement. He concludes that freezing disturbs the normal passage of ions across the membranes of the cells. In another set of experiments, he found that adult rat hearts are damaged by freezing easier than are young rat hearts. The reason is not because the adult hearts weigh more but because the membranes of the older cells are more susceptible to an influx of sodium ions from outside their membranes. Here again he has reason to believe that membranes of heart cells during freezing experienced disruptions in the passage of ions.

Both Offerijns and Rapatz, in fact, suspect that ion disruptions on the cell membrane during freezing, and perhaps during thawing as well, may be one of the major hindrances to reviving frozen hearts. On this assumption Rapatz is currently trying to offset possible ion imbalances in whole rat hearts during freezing and thawing, to see whether their chances of survival might be increased. He is putting more potassium ions into the perfusate (blood pumped through the hearts) and reducing sodium ions in the perfusate, in hopes heart cells will lose less potassium and not be flooded by sodium during freezing. Other researchers have shown cell loss of potassium during freezing. Some other researchers have noted marked improvements in frozen mammalian smooth muscle when electrolytes were readjusted in the perfusate (liquid medium), and smooth muscle is a lot like cardiac tissue.

Rapatz and Offerijns are also interested in making cryoprotectants less toxic and more effective for frozen heart tissues and cells. Exactly how cryoprotectants work is not known for sure, but recent evidence suggests they probably exert protection at the cell membrane (SN: 7/22/72, p. 54). However, Offerijns has very recent evidence that some cryoprotectants, as well as the freezing process itself, can produce adverse effects inside heart cells. The mitochondria (energy factories located in the cell cytoplasm) and the sarcoplasmic reticulum (the network of tubules that carries extracellular fluids from the outer cell membrane into the cell) are affected. In any event, Rapatz is infusing cryoprotectants into the blood that bathes the rat heart to see whether the chemicals will penetrate the muscles of the heart. He says he suspects that the tissue that lines the vascular system may not permit the protector to penetrate the muscle. The cryoprotectant he has been working with is no less than Prestone, one of the antifreeze products motorists put in their automobile radiators in the winter.

How soon basic research like that being conducted by Offerijns and Rapatz might lead to successful long-term freezing of human organs is anybody's guess. Rapatz hazards a decade. Offerijns says it "may still be in this century." Although he admits he is more optimistic than a year ago, Meryman says not before the 21st century. In the Aug. 7 JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION, the editors express doubt that frozen organ banks are a near-term possibility. "The problems associated with failure to preserve organs . . .," they write, "are quite complex and include inadequacy of perfusion coupled with the inability to deliver cryoprotective agents at the cellular levels. Furthermore, the prospects of overcoming these difficulties, as of this writing, do not appear too promising."

But cryobiologists trying to get at the basics of organ freezing and thawing, such as Rapatz and Offerijns, are confident that some day, sooner or later, they will achieve their desired aim—long-term preservation of human organs. "So many people could benefit from a new heart or kidney," Offerijns says. By that time, they hope, that immunologists will have progressed in understanding organ-transplant-rejection phenomena so that transplants are more successful. When that day comes, it is not inconceivable that thousands of hearts will be taken at autopsy and typed for perhaps dozens of immunological factors, put on dry ice, then taken out of the freezer months or years later when organ recipients have need for them. □