BEYOND THE ICE PACK

The power of cold in preservation and destruction is being explored as a source of medical techniques

The second of two articles on the art and science of cryobiology.

BY JULIE ANN MILLER

Ice packs are still prescribed for sprained ankles and for soothing an aching wisdom tooth, but low temperatures are destined for more sophisticated roles in medicine. Freezing and reuse of red blood cells is already a routine procedure, and success with other blood cells is likely. Freezing whole organs to await transplantation is a more frustrating problem, but research is progressing. And in addition to cold's preservative uses, surgeons are harnessing its destructive capacity in an icy scalpel.

Currently, red blood cells are the most valuable frozen material in medical treatment. "This technique has already come of age," says Harold T. Meryman of the American Red Cross Blood Research Laboratory in Bethesda, Md. He estimates that 200,000 units of frozen red cells are transfused annually in the United States.

The key to freezing red blood cells was the same as in the case of freezing sperm (SN: 9/16/78, p. 202). A protective chemical, such as glycerol, must be used. This procedure was used in 1950, but widespread clinical application was delayed by difficulty in removing glycerol after thawing. Washing techniques had to be developed in research laboratories operating on shoestring budgets, Meryman says. But now two manufacturing companies make automatic machinery for removing glycerol.

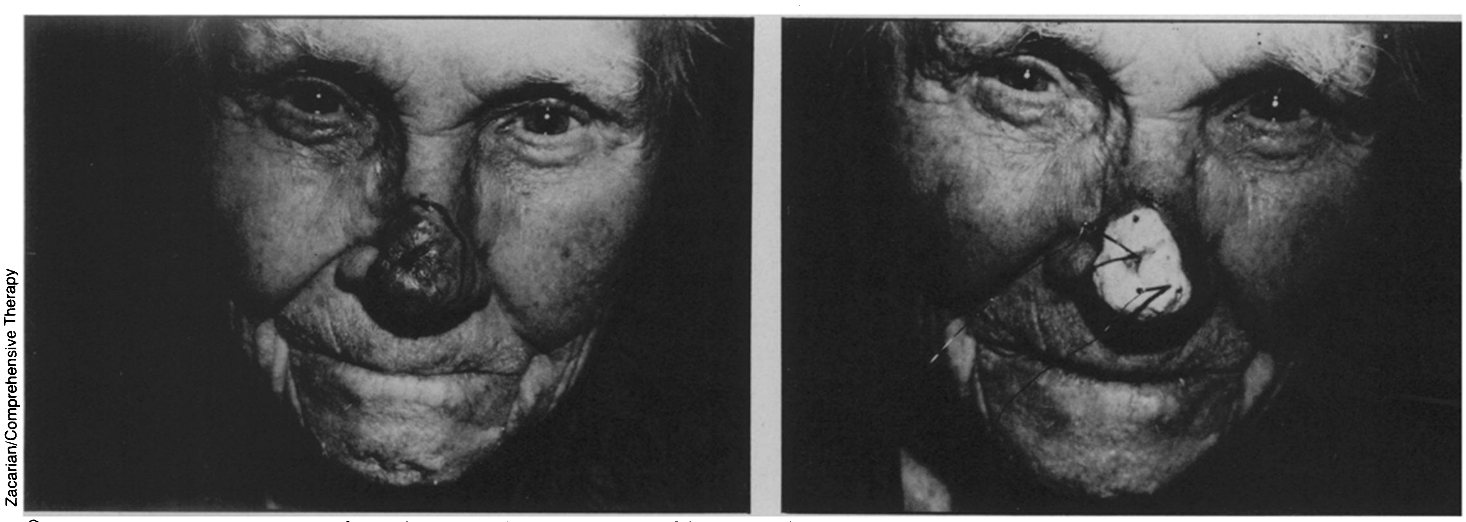
Red blood cells are currently frozen to one of several temperatures, depending on the equipment available. In some cases, the cells are stored in liquid nitrogen at −196° C and in others in mechanical freezers at −80° C. There is no evidence of deterioration, even in cells stored for more than 15 years at these temperatures. Such extreme temperatures, however, may not be necessary. Researchers are now collecting data on blood cells stored at −20° C, the temperature of an ordinary food freezer. With enough glycerol as a protectant, no special equipment is needed. The cells in suspension can be placed in a freezer and allowed to cool at their own rate. Because −20° C is not cold enough to stop metabolic activity completely, the cell medium must include materials to nourish the cells. Cells in a refrigerator can survive only about three weeks, but cells "hibernating" at −20° C can last a year, Meryman says.

The ability to store red blood cells is an important convenience. Blood banks can better match rare blood types with recipients, and blood suppliers also have less worry about peaks and lulls in blood demand and donation. In addition, thawed blood may be even better than fresh blood for the recipient. For one thing, frozen blood seems less likely than fresh blood to spread hepatitis. Researchers suggest that the disease agent is washed away during the glycerol-removing procedure. Meryman, who is currently collaborating with Japanese scientists in a clinical study of blood transfusion and hepatitis, says the early results show that freezing blood cells does not entirely prevent infection, but it may decrease the severity of the disease.

Another advantage of frozen blood is that the freezing procedure destroys most white blood cells, which carry the surface molecules (transplantation antigens) that complicate organ transplants. When the antigens on a donated organ do not match those of the recipient's white blood cells, the organ is likely to be rejected. This problem is compounded in patients, such as those on kidney dialysis, who receive many transfusions of fresh blood with different white cell types. Patients receiving multiple transfusions of frozen blood containing few white cells are less likely to become sensitized against a future kidney transplant. Frozen blood use thus eliminates an unknown factor from which a patient might suffer later.

While fragility of white blood cells is an advantage where transplants are a possibility, it is a frustration when white blood cells are required. Research on freezing white blood cells is still in the early stages, but bone marrow, the source of most blood cells, has been successfully transplanted after freezing. Meryman says the American Red Cross will begin evaluating the bone marrow preservation procedures next year to select one for routine use.

Freezing of another blood cell type, platelets, is still at the experimental stage. Platelets plug defects in blood vessel walls. They carry out their mission by sticking to the wall, disintegrating and forming a clot. As Meryman puts it, "They are programed to fall apart, rather than

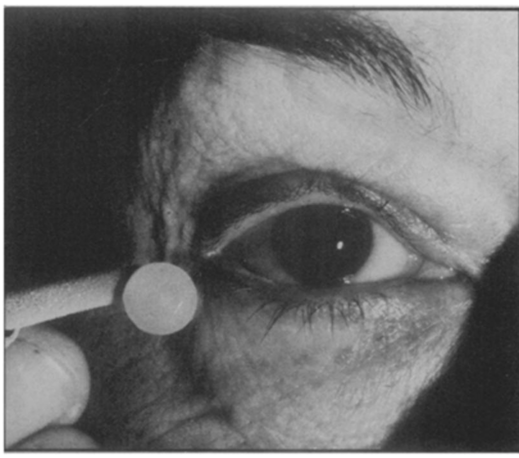


Zacarian/Comprehensive Therapy

Cryosurgery removes a cancer from the nose of a ninety-year-old patient. After three minutes, electrodes record tissue destruction.

survive." Not only are they delicate, they are also less permeable to glycerol than are red blood cells and therefore more difficult to protect during freezing. Recently, however, Arthur W. Rowe and George Dayian at the New York Blood Center succeeded in freezing and recovering human platelets suspended in a low concentration of glycerol. The researchers freeze the cells rapidly, cooling at 30° C per minute, and store them at −196° C. The frozen cells also must be rapidly thawed if they are to survive.

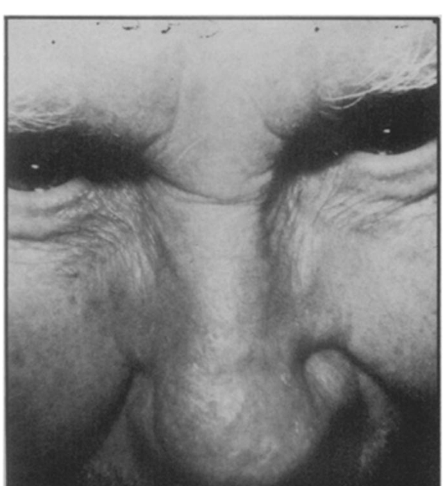
The widescale success of red blood cell transfusions is due partly to the sturdiness of those cells, but also to the simplicity of the matching problem. Generally, it is only necessary to match recipients and donors for their ABO and Rh types. Other cells, ranging from white blood cells to components of organs, present a much more difficult logistical problem. These cells have four sites for the antigens that trigger immune responses, and each site can be filled with one of more than 10,000 different antigens. Except in the case of identical twins, a perfect match is rarely possible.



National Eye Institute

Cryoprobe lifts cataract from eye.

Freezing tissue opens up new possibilities for better tissue matching. Once it is clinically possible to freeze white blood cells and bone marrow, perfect matches may be possible by having patients donate to themselves. For example, a leukemic patient whose disease is in remission could gradually build up a store of frozen cells that could be used when the condition worsens. Researchers at Oak Ridge National Laboratory speculate that one prospect might be preservation of bone marrow from healthy young people; that marrow could be reinjected decades later to rejuvenate their immune systems to fight the diseases of old age. Even under conditions where patients could not donate to themselves, a large bank of previously characterized, frozen cells would offer the best chance to find a close match.



A few months later, the area has healed.

Freezing of organs would also permit an extensive search for compatible tissue. At present, important matching procedures often cannot be done at all because they require up to two weeks, and the kidney of an accident victim, for example, can rarely be stored more than 48 hours. But the problems of freezing organs are much greater than those of storing individual cells. Organs are composed of many different cells, such as nerves, muscles and blood vessels. Each cell type is likely to have a different set of optimal conditions for surviving freezing and thawing. Thus, it is not surprising that the most successful organ transplant techniques are still rudimentary. Skin preserved in a frozen state is now used successfully to cover severe burns, but only as a biological band-aid. Most of the skin cells die, so the burn must eventually be treated with fresh grafts from intact parts of the victim's skin. "It is indeed unfortunate that the relatively successful cryobiological methods used with tissues such as cornea, or cells such as those of the bone marrow, cannot be applied with greater ease to the more solid organs such as the kidney or heart," Kenneth W. Sell of the National Institute of Allergy and Infectious Diseases told the annual meeting of the Society for Cryobiology in Tokyo.

Current research is focusing on two organ systems: the pancreas and the kidneys. Peter Mazur and colleagues at Oak Ridge National Laboratory slowly froze pancreases from rat fetuses in the presence of a high concentration of protectant DMSO (dimethylsulfoxide). The fetal pancreases, once thawed and transplanted into adult rats, were able to produce insulin and reverse experimentally induced diabetes (SN: 1/1/77, p. 4).

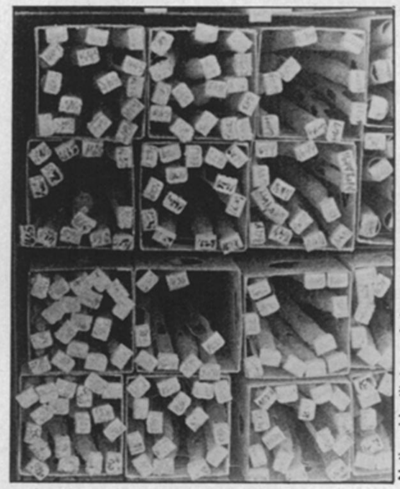
Many researchers are working on long-term preservation of kidneys, but have only occasionally reported even partial success after transplant. Meryman suspects that in preliminary experiments done at the Red Cross Laboratory blood platelets react to vascular damage in the kidney during freezing and clump, plugging the vessels.

Given that the different cell types that make up an organ are likely to have different optimal conditions for surviving freezing and thawing, David Robinson of Georgetown University says, "Unless every one of the cells of an organ can be permeated by a protective chemical, the freezing process must injure something." To make matters even worse, Robinson has found that when cells are concentrated to the levels found in solid tissue a domino effect is produced. Cells damaged by the freezing process release enzymes that destroy otherwise intact neighbors.

Robinson believes the best chance for whole organ survival may be encouragement of cell repair. He has found that some specialized laboratory-cultured cells, in a dilute suspension, can repair the damage caused by freezing to −196° C with no chemical protectant. But researchers are only beginning to examine how that may be done.

With the difficulty in freezing and reviving even a single organ, Robinson is adamant that attempts to freeze whole human beings are, at present, "a monstrous idea" and "a waste of scarce resources." Another scientist says that given the cell damage from current freezing techniques, an embalmed body would have a better chance than a frozen one of being brought back to life with, as yet, speculative methods of the distant future.

Freezing cells for medical research



National Institute on Aging

Banks of laboratory-cultured human cells preserve material for medical research. Scientists can obtain from such collections samples of cells that are already well characterized by previous investigations. Keeping cells frozen in liquid nitrogen is a far simpler, and safer, long-term operation than maintaining living cultures. Among the national cell banks is the collection at the National Institute of General Medical Sciences of cell lines useful for research on genetic disorders and that at the National Institute on Aging of cells appropriate to aging research.

While many researchers are struggling to use cold to keep tissue alive, others are taking advantage of its destructive potential. Last year surgeons, veterinarians, biologists and engineers created the American College of Cryosurgery, with 350 members.

Cryosurgery uses local freezing to destroy selected living tissues. The first account of deliberate tissue freezing was published in 1851; James Arnott reported that he froze skin with a salt-ice mixture. Since then solid carbon dioxide, liquid nitrogen and cold air blasts have been applied to destroy benign tumors and premalignant skin conditions. And with modern instruments, such as the cooling probe, it became possible to use freezing to treat a wider variety of medical conditions. In the cooling probe liquid nitrogen circulates through a hollow metal tool, well insulated except at the tip. With such a probe surgeons can control the temperature down to $-196°$ C and apply cold to any point accessible to the probe.

Freezing tissue has several advantages over traditional surgery: The profound cold anesthetizes the area and controls bleeding by injuring small blood vessels. It is advantageous cosmetically because cryosurgery rarely produces scar tissue. More than one-third of the cases of common skin cancer are now treated with freezing techniques. And ophthalmologists use the technique in 90 percent of cataract extractions. Freezing is also the routine procedure in treating trichiasis, a condition in which an eyelash turns to grow against the eye.

For some surgery, a spray of liquid nitrogen is more effective than the probe. Purnendu Dutta and Andrew A. Gage of the Veterans Administration Hospital in Buffalo, N.Y., report yet another technique, one for destroying large volumes of liver. They cover the liver with a dacron velour cloth impregnated with lubricating jelly and pour liquid nitrogen onto the cloth. In experiments with dogs this method has prevented cracks and, in turn, a bleeding problem on the frozen liver surface.

A new way to monitor the freezing process is another recent technical development. Currently, surgeons use a thermocouple needle to measure the temperature of a tissue as it is chilled. However, as tissue freezes, its electrical resistance drops. Setrag Zacarian of Albany Medical College and Michael Savic of Western New England College have developed an instrument that can measure that resistance during surgery. "It is a much more rapid method of determining cryodestruction," Zacarian says.

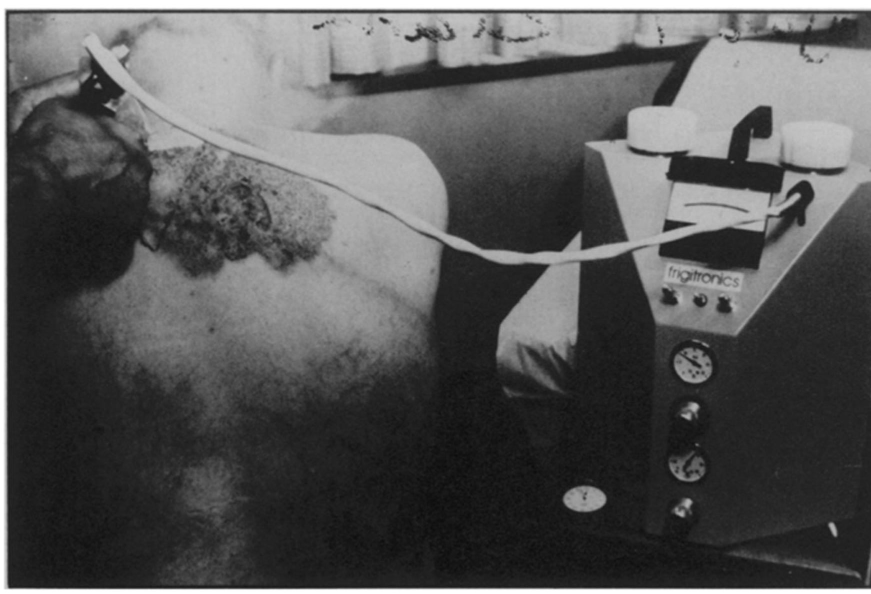
At the Tokyo meeting, researchers reported clinical studies on cryosurgery of cancers of the head and neck, mouth, skin and reproductive and lower digestive tracts. In general, cryosurgery was most successful for small tumors, and several investigators recommended combining it with radiation treatment or chemotherapy. Animal studies were also reported on cryosurgery for cancer of the upper digestive tract and malignant melanoma. In addition to cancer treatment, cryosurgery is being investigated for medical problems such as mouth ulcers, ingrown hair infections and internal hemorrhoids.

Zacarian, working with Fritz Fraunfelder of the University of Oregon and Crowell Beard of San Jose, Calif., is examining 450 cases of patients with eyelid cancers treated by cryosurgery. They are finding "very acceptable cure rates," Zacarian says. Cryosurgery is already used routinely to treat eyelid cancer in cattle. That skin cancer is a problem among white-furred Hereford cattle who, like fair-skinned people, are sensitive to the sun.

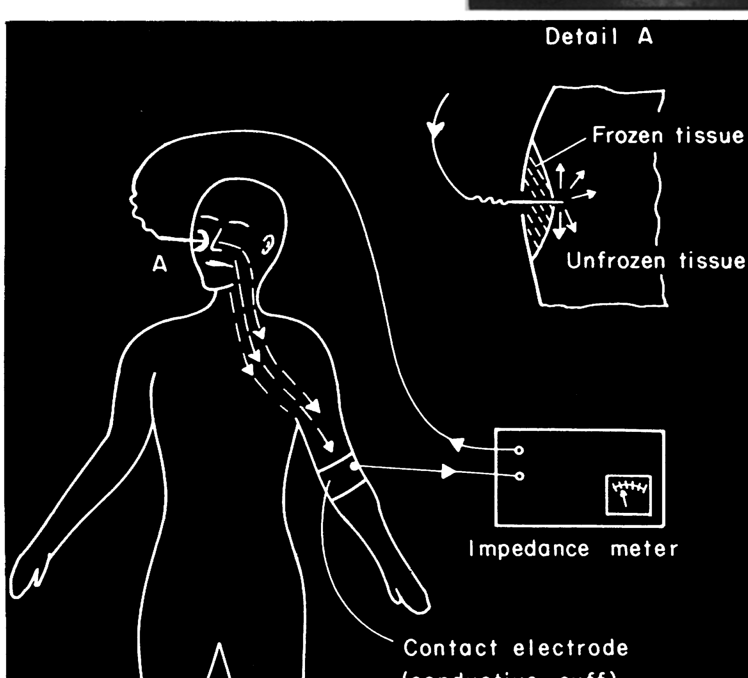
Perhaps the most exciting prospect for cryosurgery is the suggestion that it can turn the body against a cancer. Animal and clinical studies have provided preliminary evidence that destruction by freezing initiates an autoimmune response. In one case, regression of cancer that had spread throughout the body followed cryosurgery of a primary prostatic tumor.

In animal studies, researchers have detected, after cryosurgery, antibodies in the blood that specifically react with the tissue destroyed. One possible explanation is that, during freezing, cell surface molecules are changed or previously unexposed components are released and the immune system responds to those complanation, researchers are working to enhance that tantalizing cryoimmunologic response.

So cryobiology is likely to remain hot as an area of medical research, at least until cancer can be chilled right out of the body and until human parts can be pulled from the freezer and reinstalled. □



Frigitronics, Inc.



Savic and Zacarian/J. of Derm. Surg. and Onc.

Cryosurgical unit feeds liquid nitrogen to closed probes or spray cones (above). The change in electrical resistance of frozen tissue signals cryodestruction in a new method for monitoring the surgery.