BIOLOGY

Live Cells Frozen Alive

The science of freezing cells at extremely low temperatures is expected to lead to preserving samples of species about to become extinct, Ann Ewing reports.

➤ PUTTING LIFE, even human beings, into an extreme deep freeze for preservation by suspended animation may not be too far in the future.

For living cells it has already been done. Biological scientists have borrowed a trick from the physicists and plunged living cells directly from room temperature to temperatures 345 degrees below zero Fahrenheit. When thawed equally as fast, the red blood cells are not damaged.

Using low temperature techniques, scientists may in the future preserve samples of species about to become extinct, store cell or tissue cultures and antibiotic-producing organisms, and stop life processes, in disease as well as in health.

Many investigators today believe that the death of cells from freezing results not from the freezing process itself but rather from a slow freezing, during which ice crystals can form, and even more from a slow thawing, when ice crystals can also be formed.

Cryogenics is the field of physics dealing with how matter reacts at temperatures near absolute zero or 459.7 degrees below zero Fahrenheit. There have been tremendous strides in the last 10 years in applying cryogenics to missiles, electronics and gas liquefaction.

However, only in a few laboratories are scientists investigating the possibilities of cryobiology, or biological reactions at very low temperatures. One obvious application is in the preservation of blood and tissue for treating injuries, either in peace or war.

Basic And Applied Research

The U.S. Navy and the National Institutes of Health are among the agencies involved in basic and applied research on biological reactions at very low temperatures.

Until recently, man's interests in biologic freezing were focused primarily on defense against it, such as the prevention and treatment of frostbite, according to Dr. Joseph F. Saunders, head of the medicine and dentistry branch, Office of Naval Research.

Very little attention was given to the possible beneficial effects of cold, particularly as a practical tool in biologic research. Scientists have, however, studied the effects of temperature reduction in warm-blooded animals, such as occurs either during hibernation or experimental hypothermia. The latter is the induced lowering of the body temperature that is now being used more and more for patients undergoing certain kinds of surgery.

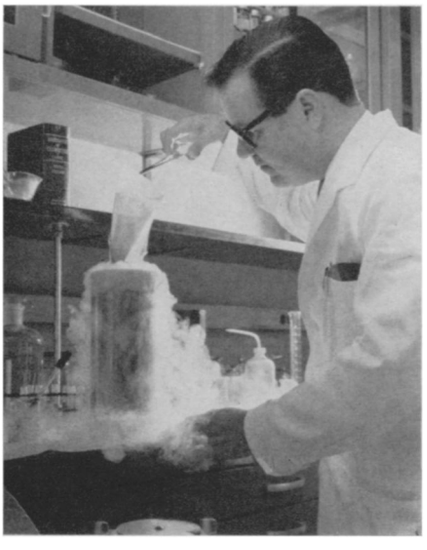
The effects of freezing stem from two factors, the formation of ice crystals and the dehydration, or water loss, accompanying the crystal formation. The slower the freezing rate, the larger the ice crystals formed. As the rate of freezing increases, the ice crystals become more numerous and smaller, until a point is reached when they finally vanish entirely. The resulting ice is solid and glassy.

To remain frozen without the danger of crystal formation, biologic materials must be stored below the recrystallization point of ice, which is 200 degrees below zero Fahrenheit. Protective substances can be added and glycerol, glucose and polyvinylpyrrolidone are among those used to help protect blood.

The Linde Company, a division of Union Carbide Corporation, is developing a freeze-thaw method of processing human blood for indefinite storage at liquid nitrogen temperature, which is minus 345 degrees. The goal is to achieve a simple, economical and speedy process of blood preservation, storage and transfusion without any intermediate steps in the process from donor to recipient.

Equipment has been developed in which a pint of blood can be frozen or thawed in one minute or less. The thawed blood can be stored in an ice bath at 32 degrees Fahrenheit as long as three weeks.

Although cryobiologic research has been mainly concerned with finding satisfactory blood-preservation techniques, there have been some investigations on the effects of low temperature on other biological materials. Spermatozoa, for instance, were successfully frozen and revived as far back as 1938.



DEEP-FROZEN BLOOD—Red blood cells frozen at 345 degrees below zero Fahrenheit are removed from the freezing flask by Robert C. Scheno, biologist at the division of biologics standards, National Institutes of Health, Bethesda, Md.

In 1952, a freeze method for preserving fertilized rabbit eggs was developed and it was found that one percent of the eggs survived after thawing. Although this is not a high rate, it is enough to show that even a cell in such an unstable state does not necessarily die when exposed to low temperatures.

Problems Are Complex

The problems involved in freezing such highly developed organisms as animals, including man, are extremely complex. Until recently, it was thought that a rat could not survive cooling to an inside body temperature below 69 degrees Fahrenheit, at which point respiration and heart beat stop. However, the emperor penguin not only supports life but "contrives to incubate eggs and care for its chicks in Antarctic temperatures as low as 76 degrees below zero Fahrenheit," Dr. Saunders noted.

Laboratory experiments have shown that rats, mice and dogs can be revived after cooling to deep body temperatures of 32 degrees Fahrenheit. Rats so treated showed no impairment of either memory or learning capacity.

In the future, Dr. Saunders foresees the following possibilities of biological use of low temperatures:

Preserving such large specimens as the brains of vertebrates for study, with a minimum of chemical fixation.

Preserving and storing live bacterial and cell or tissue cultures.

Long-term banking of antibiotic-producing organisms, eliminating today's necessity of "starting again from scratch."

Arresting chemical and other life processes in disease as well as in health. Important enzymes, for instance, might be preserved without losing their functions.

Studying now unidentifiable but important intermediates in biochemical reactions.

Preserving today's generations of organisms for later comparison with future generations, and also tissues affected by today's diseases for comparison with diseased tissues several years from now.

And far in the future, Dr. Saunders foresees the preservation of live plants and animals to prevent extinction of a species. Such an application, he said, cannot be dispelled as "being impossible or fantastic," because, theoretically, life virtually does experience a temporary halt at temperatures around and below the liquid nitrogen range.

A pioneer in the study of the effects of low temperature on living cells is Dr. Basile J. Luyet, a Jesuit priest who is director of the American Foundation for Biological Research, Madison, Wis. In 1949, he showed that 70% of the red cells in smears of oxa-

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Woodger—*Harper,* 200 p., paper, $1.50. Reprint (1933).

THE NATURE OF ATOMS AND MOLECULES: A General Chemistry—Ewing C. Scott and Frank A. Kanda—*Harper,* 765 p., illus., $8. Textbook emphasizing chemical correlations, intended for college students who have serious interest in chemistry.

THE NEW SCIENCE OF SKIN AND SCUBA DIVING—Conference for National Co-operation in Aquatics, Alex A. Maleski, Chmn.—*Assn. Press,* rev. ed., 208 p., illus. by Andre Ecuyer, $3.95; paper, $2.95. Replaces original (1957) official text.

NINE PLANETS—Alan E. Nourse—*Pyramid,* 288 p., illus. by Mel Hunter, paper, 75¢. Reprint (1960).

NUCLEAR SUBMARINE SKIPPERS AND WHAT THEY DO—Commanders George P. Steele and Herbert J. Gimpel, USN—*Watts, F.,* 140 p., $3.95. Includes glossary and index.

1,000 ANSWERS TO QUESTIONS ABOUT PHOTOGRAPHY—Robert L. McIntyre, Ed.—*Grosset & Dunlap,* 248 p., photographs, paper, $1.95. Reprint (1959), formerly "1,000 Photo Questions Answered by Experts."

1001 QUESTIONS ANSWERED ABOUT BIRDS—Allan D. and Helen G. Cruickshank—*Grosset & Dunlap,* 291 p., photographs by author, illus. by James Macdonald, paper, $1.75. Reprint (1958).

PRINCIPLES OF PHYSICS—Earnest S. Greene—*Prentice-Hall,* 806 p., diagrams, $9.75. Text intended for physics course at the liberal arts and premedical level.

THE REAL NUMBER SYSTEM IN AN ALGEBRAIC SETTING—J. B. Roberts—*Freeman,* 145 p., diagrams, $3.50; paper, $1.75. Textbook intended to acquaint student with the basic facts of an important mathematical system, also of cultural value to nonscience students.

ROBERT BOYLE: Founder of Modern Chemistry—Harry Sootin—*Watts, F.,* 133 p., illus. by Gustav Schrotter, $1.95. Biography for young people.

SCIENTISTS: Their Psychological World—Bernice T. Eiduson, foreword by Harrison Brown—*Basic Bks,* 299 p., $6.50. An examination of the nature of technically trained people, dealing with parental backgrounds, interests, aptitudes, personality, intelligence and other motivating factors of 40 contemporary American research scientists.

SEMIMICRO LABORATORY EXERCISES IN GENERAL CHEMISTRY—J. Austin Burrows, Paul Arthur and Otto M. Smith—*Macmillan,* 3rd ed., 306 p., illus., paper, $4. Includes more quantitative experiments and experiments introducing modern techniques.

THE SMALL GROUP: An Analysis of Research Concepts and Operations—Robert T. Golembiewski—*Univ. of Chicago Press,* 303 p., $6. Reviews research in small-group analysis, and points out its useful relevance to students of behavior.

SOUTH AMERICA A TO Z—Robert S. Kane—*Doubleday,* 370 p., photographs, maps, $4.95. A practical guide to 14 Latin American countries, for the tourist who also wants to know something about their historical and political background.

SPACE BIOLOGY: The Human Factors in Space Flight—James Stephen Hanrahan and David Bushnell—*Science Editions,* 285 p., photographs, paper, $1.95. Reprint (1960).

THE STARS: A New Way to See Them—H. A. Rey—*Houghton,* rev. ed., 160 p., illus., $6. Guide to the constellations for beginners. Jacket unfolds into 22x26-inch map.

THEORY OF THE TRANSMISSION AND PROCESSING OF INFORMATION—A. G. Vitushkin, transl. from Russian by Ruth Feinstein—*Pergamon,* 206 p., $15. Research monograph, attempts to define mathematically the construction of tables for functions, a concept important in the automatization of programming.

TIME'S ARROW AND EVOLUTION—Harold F. Blum—*Harper,* 220 p., illus., paper, $1.65. Reprint of 2nd ed. (1955).

UNIFIED ORGANIC CHEMISTRY—Charles A. MacKenzie—*Harper,* 586 p., illus., $8.50. Text combines aliphatic and aromatic compounds in an order which emphasizes functional groups.

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lated ox blood remained alive when cooled at a rate of about 200 degrees per second by immersion in liquid nitrogen and then rewarmed at about the same rate.

In 1950, Dr. A. U. Smith of the Institute for Medical Research in England discovered that a 15% glycerol solution protected rabbit and human red blood cells from death during freezing at temperatures of more than 100 degrees below zero Fahrenheit.

The aim of current research, Dr. Harold Meryman of the Naval Medical Research Institute, Naval Medical Center, Bethesda, Md., told SCIENCE SERVICE, is to find an additive to protect frozen blood that does not have to be removed before the blood can be used for transfusion. Transfusions could then be started within 90 seconds instead of the more than an hour now needed to prepare the blood.

He said that Dr. Audrey Smith and her co-workers at the Mill Hill Laboratory near London were studying the freezing of whole organs and whole animals.

Dr. Meryman said the current belief was that the stage for damage from cold was set during the freezing process, but that the final blow making the damage irreversible occurred during thawing.

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FISHERIES

No Bones About It, Machine Does the Job

➤ DON'T LIKE FISH because of the bones? How about a machine that debones them?

The U.S. Bureau of Commercial Fisheries Review, 24:15, 1962, claims it is now possible to design and build a machine that will detect and reject those harmful fish bones.

Research by the Bureau has been carried on since 1954 to find such a machine. When the results were in, the scientists report that there are no bones about it, the machine really works.

Fish are relayed in front of an X-ray unit which detects the bones, even when the fish are frozen. An electronic flash forms an image of the fish's interior on a fluoroscopic screen. The bones cause a change in energy levels on the screen which can be picked up and relayed to a photoelectric unit. This unit can control a rejecting device which de-ribs the fish.

The device works best on thin slices but Bureau scientists are confident that it can be improved for commercial use.

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