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Boston University School of Medicine

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Boston University
Coronary artery disease this year will claim the lives of more than 600,000 Americans. BUSM scientist Carl Franzblau, Ph.D., has for 10 years headed a research project that examines the role of arterial connective tissue components in this disease. See story on page 2.

**Shelf-life of blood extended through freeze-preservation, rejuvenation process**

Researchers at the Naval Blood Research Laboratory at Boston University School of Medicine have developed methods of preserving and rejuvenating blood cells that are expected to revolutionize the nation's blood-banking system. For instance, the researchers have developed:

- a freeze-preservation technique that enables red blood cells, normally able to be preserved for a maximum of five weeks, to be preserved for up to 10 years;
- methods to biochemically rejuvenate outdated red blood cells that ordinarily would be discarded after liquid storage;
- methods to boost the oxygen-transport capability of red blood cells by 150 to 200 percent, to allow the cells to be used for special medical purposes.

The procedures, the result of nearly 25 years of research, already are being put to use. The laboratory's techniques for freeze-preservation of red blood cells has been adopted by numerous civilian and military blood banks throughout the country, including the blood bank at University Hospital, a principal teaching hospital of BUSM. More recently, the rejuvenation technique has been adopted by the American Red Cross in Boston and by several hospitals.

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**BUSM researchers advance treatment of male impotence**

Occasional impotence, or the failure to achieve and maintain an erection, is part of a normal sexual life. At some time in their lives, most males experience impotence due to anxiety, stress, guilt, depression or an excess of drugs or alcohol. For some males, though, impotence is a chronic and disturbing problem. Boston University School of Medicine researchers have been studying the problem for some time and have made important contributions regarding the diagnosis and treatment of those having impotence.

Robert Krane, M.D., chairman of the School’s Department of Urology, chief of the Department of Urology at University Hospital and co-director of the New England Male Reproductive Center at the Hospital, is studying this problem with Mike Siroky, M.D., also co-director of the Male Reproductive Center, and Terry Payton, R.N. Siroky also is an assistant professor of urology at BUSM.

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Connective tissues may provide key to understanding atherosclerosis

More than 600,000 Americans will die this year from coronary artery disease. The primary cause of this disease is atherosclerosis, which occurs when the inner walls of the arteries thicken and narrow, obstructing the blood supply to vital organs. What follows can be death of heart tissue (myocardial infarction), death of brain tissue (stroke, or cerebral infarction), or, occasionally, gangrene of the hands and feet.

High blood cholesterol levels are believed to be responsible for much of the accumulation of fat that thickens the inner arterial walls. This accumulation eventually leads to the formation of atherosclerotic plaque, thick, crusty material primarily consisting of hardened arterial connective tissue proteins known as collagen and elastin, lipids, calcium and phosphate. Lipids, which are fatty substances that make up the structure of living cells, also accumulate in arteries blocked with plaque.

For unknown reasons, smooth muscle cells sometimes over-proliferate, resulting in the excessive production of collagen. As more connective tissue is produced, it may calcify or harden, and eventually close the lumen of the artery through which blood flows. As a result, the blood supply is severely hampered and the heart does not receive enough oxygen. Acute pain then sets in, and a myocardial infarction occurs.

Franzblau and his team of co-investigators have been looking for clues to help them better understand the nature of these arterial connective tissues. "It is our belief," said Franzblau, "that by studying the cells involved in atherosclerosis, we should gain new insights into the disease process.

"We do know that atherosclerosis is multifactorial," Franzblau continued. "Lipids, lipoproteins, calcium and glycoproteins, as well as the connective tissue proteins, collagen and elastin, are all components in the final debilitating plaque that forms the latter stages of the disease."

But what triggers abnormal proliferation of smooth muscle cells in the arteries? How can these events be monitored? Are there any inherent protections against atherosclerosis? To answer these and many other questions about arterial connective tissue in vivo, Franzblau and his group have developed cultures of arterial smooth muscle cells that are capable of producing large quantities of connective tissue proteins. The cultures, which are derived from the artery of a young rabbit or calf, are fed several nutritive substances. The cells, grown in sterile dishes, multiply continuously and accumulate.

Biochemists at the School of Medicine, who conduct the bulk of their work in the School's Housman Medical Research Center, also have been evaluating the role of estrogen in the formation of connective tissue proteins.

Gail Sonenshein, Ph.D., an assistant professor of biochemistry and an Established Investigator of the American Heart Association, has been researching the effects of estradiol, the major human female hormone, on a smooth cell culture system.

"Using two culture systems — one fed estrogens and one not — we study the regulation of the events involved in the production of collagen," she explained. "What we have observed is that after estrogen is introduced into the system, collagen production decreases."

To fully understand how these events are regulated, Sonenshein dissects cells to examine their nuclei and to observe what happens to the hereditary factors, chromosomes and genes. Here the messenger molecules that carry the coded genetic information are produced and travel to the cytoplasm. It is in the
cytoplasm, or the outer portion of the cell, where proteins are produced.

"Further research and evaluation in this area may help us gain insight into the prevention or regression of atherosclerosis, and whether other types of hormones affect the cells," Sonenshein said. "In the future, we will use cloning technology with recombinant DNA molecules."

Franzblau emphasized the collaborative nature of the research in atherosclerosis taking place at the Medical Center. "We don't operate in a vacuum," he said. "We have had a long-term collaboration with William Hollander, M.D., a BUSM professor of medicine, physiology and biochemistry, who is the principal investigator of a large research program in atherosclerosis."

Working with members of the Cardiovascular Institute at BUSM, Franzblau has been able to simulate hypertension in the laboratory. Cells are placed in a newly-designed pressure machine that mimics a hypertensive environment. By simulating an in vivo act, Franzblau explained, one can closely monitor and evaluate the effects on the cells.

Franzblau and other members of the Department of Biochemistry also plan to develop an aging smooth cell culture system to study the effects, if any, of aging on the pathogenesis of atherosclerosis.

"Although there are inherent shortcomings in all cell culture systems when one tries to relate the data to the in vivo situation, we are optimistic that new insights into our understanding of the development of atherosclerosis will be forthcoming," Franzblau concluded.

Other members of the BUSM Department of Biochemistry who are investigators in the connective tissue and smooth muscle cell research project, along with Franzblau and Sonenshein, are Lily Salcedo, M.D., Ph.D., and Paul Toselli, M.D., Ph.D., both assistant professors of biochemistry, and John Beldekas, Ph.D., a research associate in the Department. Graduate students and postdoctoral fellows also contribute significantly.

Herbert M. Kagan, Ph.D., and Karl Schmid, Ph.D., both professors of biochemistry, are investigating other aspects of the development of atherosclerosis.

—Susan B. Saperstein

Suggested Further Readings


Blood preservation...

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"We are involved in areas of blood research that have dramatically improved the blood-banking system in both the military and civilian community," said Capt. C. Robert Valeri, MC, USN, an adjunct research professor of medicine at BUSM, who has served as director of the NBRL since 1963.

Charles P. Emerson, M.D., an emeritus professor of medicine at BUSM, is principal investigator for the facility, which provides a vital link for those doing blood research at numerous medical schools and hospitals in the Boston area. Others doing research at the NBRL include Eugene N. Serrallach, Ph.D., a research associate in medicine at BUSM; Richard Dennis, M.D., an assistant visiting surgeon at University Hospital; and Cmdrs. Anthony J. Melaragno, MC, USN, and Allen Holland, MC, USNR, and Lt. Craig Thompson, MC, USNR.

The history of the NBRL, internationally known for its research on the preservation of blood and blood products, dates back to 1956 when the laboratory was part of Chelsea Naval Hospital in Boston. It was established as a separate unit of the hospital in 1965 by the Navy's Bureau of Medicine and Surgery, and it remained at the hospital until the installation was closed in 1976. The laboratory then moved to temporary quarters in BUMC's Talbot Building pending renovation of its present site on Albany Street, adjacent to the Medical Center. The building on Albany Street was donated to Boston University by New England Nuclear Corporation. The Medical Center, in turn, deeded the building to the Navy for its frozen blood program. The government-owned laboratory has been administered by the University under an Office of Naval Research contract since 1979.

Researchers at the laboratory,
supported by the Bureau of Medicine and Surgery’s Naval Medical Research and Development Command, since 1956 have been developing ways in which the shelf-life of blood and its components can be extended. Working with blood from animals and humans, the researchers have devised a system in which a universal blood type, such as O− or O+, can be biochemically modified (rejuvenated), frozen with the cryoprotectant, glycerol, then stored at −80°C, nearly triple the coldness of a home freezer, for transfusion at a later date when the red cells may be unavailable through the regular blood-banking system.

The entire process takes place in a routine polyvinylchloride plastic bag that was designed by the NBRL researchers to simplify the process and ensure that the red blood cells would not become contaminated during the rejuvenation and freezing procedures.

When needed, the frozen red cells are thawed in a warm water bath and then washed to remove the rejuvenation and glycerol solutions before being transfused into a patient.

Among the researchers’ findings are that nonrejuvenated frozen red cells (red cells frozen within three to six days of collection) can be stored at −80°C for at least 10 years. They also have found that indated and outdated-rejuvenated red cells can be frozen for up to four years at −80°C.

In addition to preserving the red cell component of blood, the researchers are striving to improve the preservation of platelets and plasma-clotting proteins that are involved in blood clotting; white cells that help the body fight infection and disease; lymphocytes that play a key role in the body’s immune system; and hematopoietic stem cells that produce red and white blood cells and platelets.

With further research, we expect to be able to expand the present period during which platelets can be frozen and stored at minus 80 degrees Centigrade by at least two years with a solution of dimethylsulfoxide,” Valeri said.

“It has been almost 25 years since freeze-preservation technology was first instituted as a means to supplement the liquid blood-banking system. During this time, freeze-preserved red blood cells have been used to supply rare red cells; red cells lacking antigens that commonly sensitize recipients; the donor’s own red cells for anticipated future use; and red cells free of white cells, platelets and plasma proteins to prevent transfusion reactions,” Valeri said.

The researchers also found that red blood cells, though viable for several weeks in the liquid state, do not transport oxygen normally after the first week. They developed methods to prepare and preserve red cells with a normal or improved oxygen delivery capacity by using biochemical modification and preservation in the frozen state for at least four years.

“Red cells with super-normal (150 to 200 percent of normal) oxygen transport function have been used to treat hypothermic patients in hemorrhagic shock, patients with fixed cerebral or coronary blood flow, and patients undergoing extracorporeal circulation,” Valeri said.

—Marge Dwyer

Suggested Further Readings

Male impotence . . .
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"Short-term situational impotence is quite common," said Krane, "but doctors are discovering more and more men whose problem is not psychological in nature, as was long believed, but has physical origins." This awareness is helping doctors find new approaches to treatment.

Doctors have long known that an erection is controlled by stimulation of the nerves of the parasympathetic nervous system (one of the two divisions of the automatic nervous system that regulates such "automatic" activities as heart action). "We have found, however, that the sympathetic nervous system is also involved and we are attempting to prove this by studying the effects of drugs on the neurophysiology of erection in the dog," Krane said. "We know that certain drugs, such as those taken for high blood pressure, may interfere or block nerve receptors and lead to impotence. One goal of this research is to elucidate the neurophysiology of impotence and to develop drugs that could treat this problem."

In addition to certain drugs, such as those that lower blood pressure, anti-depressants, sleeping pills, tranquilizers, and alcohol, there are many other physical causes of impotence. Diabetes, for instance, can cause penile atherosclerosis and impede blood flow to the erectile tissues, resulting in impotence. Other causes are paraplegia, structural abnormalities and injuries to the genitals, surgery for cancers of the pelvic area, Peyronie's disease (accumulation of scar-like tissue in the penis), and malnutrition. Hypertension and smoking may also be risk factors, as they narrow the arteries, restricting blood flow to the penis.

These physical causes account for 48 percent of impotence cases, with the remaining 52 percent due to psychological causes. Of the physical causes," explained Krane, "21 percent are due to vascular problems in which blood flow to the penis is insufficient to permit erection, 19 percent are neurological conditions due to diseases or nerve injury, and 8 percent are endocrinological, due to abnormal amounts of hormone.

"It appears that impotence of 'old age' is actually due to vascular causes," said Krane, "but vascular insufficiency can also affect relatively young men, say men in their forties. Studies by Kinsey (the famous researcher on sexuality), seemed to show that with each decade of age, there are marked increases in the rate of impotence, but age is not necessarily the cause." Some loss of potency may occur with advancing age, but in a healthy male, the capacity for an erection should persist well into the seventies.

Clinical research by Krane and Siroky has lead to the development of a test to diagnose vasculogenic impotence. An erection occurs when blood fills the corpora — the two rod-shape bundles of spongy tissue that constitute most of the penis. When blood flow to the penis is impeded due to such things as pelvic surgery, arterial disease, injury, or structural abnormality, impotence results. "By measuring blood pressure in the corpora before and after leg exercise, we can detect abnormalities and obstructions of the penile arteries," Krane said. "Thus, penile blood pressure should be monitored routinely." This can be done with a special blood pressure cuff fitted to the penis and a Doppler probe, an instrument that reflects ultrasound waves from blood circulating in the penile arteries.

"One possible solution to obstructed penile arteries is angioplasty, or stretching the artery at the point of obstruction, thereby letting the blood flow more freely to the penis," said Krane. Lack of sufficient blood to the penile arteries may be due to a mechanism in the body that diverts blood from the penis to the legs in what is called "pelvic steal." "We've observed many patients who were able to obtain erections while lying on their backs, but lost them when they moved their thigh or buttock muscles," Krane said.

Krane and Siroky are also using x-rays to observe any changes that may have occurred in the arteries, and to see whether or not the arteries are normal. A contrast media is injected into the arteries that supply the penis, thereby enabling the researchers to observe these changes.

Other vasculogenic research involves studying the mechanism of impotence after therapeutic radiation. Radiation for pelvic tumors often leads to impotence. "By the use of radionuclide scanning techniques, we may be able to diagnose vasculogenic impotence. Hopefully, these radiation studies will lead to better use of radiation to prevent the occurrence of impotence," Krane said.

Future research will involve studying the role of kidney failure and anti-hypertension medication. "Both of these cause impotence, and by neurological testing and use of the Doppler probe, we hope to understand the mechanism by which they cause impotence," Krane said.

"Besides being due to vascular problems, impotence also may be caused by hormonal abnormalities more often than previously believed," Krane said. In the past, men with impotence were told to see a psychiatrist, but due to recent advances in endocrinology, doctors are restructuring their approach. Krane and Siroky's research in this area involves testing the levels of hormones that are associated with impotence — testosterone, prolactin, and estradiol. "In a recent study of 260 patients, 21 were found to have low testosterone levels," he said.

In addition, an excessive amount of prolactin, produced by the pituitary gland, may interfere with erection. "We are studying specific treatments with new drugs and treating some
cases with appropriate hormones," said Krane.

If sex therapy, vascular surgery or hormonal treatment fail to restore potency, one last remedy is a penile implant, an artificial device that produces an erection. The School of Medicine researchers were the first in the United States to implant a new prosthetic device designed by Udo Jonas, a German physician. In fact, Krane and Siroky evaluated the Jonas implant for Food and Drug Administration approval.

The Jonas implant consists of a pair of flexible cylinders made of silicone rubber surrounding an inner core of silver wire. Because of the flexibility of the silver core, the penis may be positioned manually in either a resting state or at an erect angle.

Implantation, which takes about 30 minutes, involves placing one rod in each corpus. "We've had an excellent success rate with the Jonas," said Krane. "Out of the first 73 we implanted, there were only five complications, which resulted in removing three; the rest are functioning well."

Another device that Krane and Siroky implant is "the pump" or an inflatable prosthesis. This involves inserting two expandable, balloon-type cylinders into the corpora. The cylinders are connected by tubing to a small reservoir implanted near the bladder, and to a tiny pump, inserted in the scrotum. When squeezed, the pump forces saline solution from the reservoir into the cylinders in the penis, resulting in an erection. After ejaculation, the solution flows back into the reservoir.

Patients who receive implants come to the Medical Center from all over New England. Most patients are between the ages of 40 and 60, with the majority between 50 and 59. Very few are under age 30, unless they've had some kind of injury. "Not everyone can be helped; for example, those incapable of orgasm."

Those whose problem is psychological require sex therapy. For those patients who have received a penile implant, restoration of their potency results in increased self-esteem. In fact, 65 to 75 percent of our patients report a greatly improved self-image," Krane said.

To further aid impotent patients, Krane and Siroky have produced a medical teaching film for physicians who implant the Jonas device, and they have written two books for the profession. The first, Clinical Neurourology, was published by Little Brown and Company in October, 1979, and the second, Male Sexual Dysfunction, also to be published by Little Brown, will be out next year.

— Liza Hubbard

Suggested Further Readings