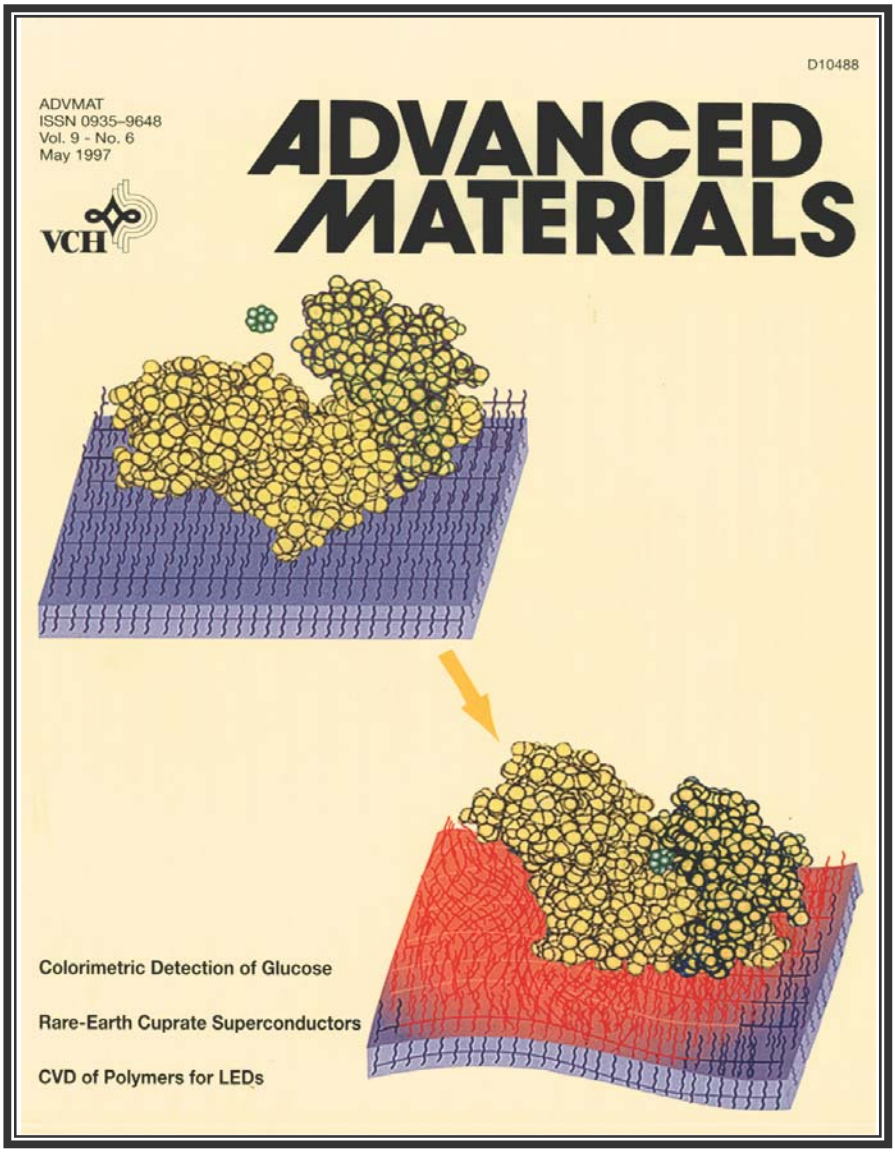
Medical Applications of Non-Medical Research



Applications Derived from BES-Supported Research and Research at BES Facilities

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APPLICATIONS DERIVED FROM BES-SUPPORTED RESEARCH AND RESEARCH AT BES FACILITIES

OFFICE OF BASIC ENERGY SCIENCES OFFICE OF ENERGY RESEARCH U.S. DEPARTMENT OF ENERGY

The Office of Basic Energy Sciences. The Basic Energy Sciences (BES) program within the U.S. Department of Energy's Office of Energy Research is one of the Nation's foremost sponsors of fundamental research in materials sciences, chemical sciences, geosciences, plant and microbial sciences, and engineering sciences. The program funds more than 2,400 researchers at 200 institutions nationwide and supports 17 major national user facilities. The BES program underpins the DOE missions in energy and the environment, advances energy-related basic science on a broad front, and provides premier national user facilities for researchers from academia, industry, and government laboratories.

The results of BES research and the availability of major user facilities also impact other research areas of importance to the Nation. One such research area of increasing importance is that of medical sciences. The following stories illustrate how BES research and major user facilities have impacted the medical sciences in the selected topical areas of disease diagnosis, treatment (including drug development, radiation therapy, and surgery), understanding, and prevention.

1. DISEASE DIAGNOSIS

Thin-Film Lithium Batteries for Biomedical Applications (ORNL). Through its BES program, the Oak Ridge National Laboratory (ORNL) has developed high-performance thin-film lithium batteries for a variety of technological applications. These batteries have high energy densities, can be recharged thousands of times, and are only 10 microns thick. They can be made in essentially any size and shape. Recently, Teledyne licensed this technology from ORNL to make batteries for medical devices including electrocardiographs. In addition, new "textured" cathodes have been developed which have greatly increased the peak current capability of the batteries. This greatly expands the potential medical uses of the batteries, including transdermal applications for heart regulation.

Positron Emission Tomography (BNL). The February 19, 1996, issue of *Chemical and Engineering News*, a publication of the American Chemical Society, featured Positron Emission Tomography--a medical imaging technique--as its cover story. The technique can track chemical reactions in living tissues and merges chemistry with biological imaging. Its strength has been in studies of the brain where there has been significant progress in investigations of drug addiction,

aging, mental illness, and neurogenic disorders. Positron Emission Tomography (PET) had its genesis in hot-atom chemical research supported by the Chemical Sciences Division of the Office of Basic Energy Sciences. Through this research it was learned, over many years, how to prepare short-lived positron emitters such as ^{18}F whose half-life is 110 minutes. In 1975, the molecule [^{18}F]fluorodeoxyglucose was successfully synthesized at Brookhaven National Laboratory (BNL) and set the stage for Positron Emission Tomography of the human brain. There are about 250 PET facilities world-wide, including 60 in the United States. Many of the institutes, medical schools, and universities throughout the world using PET have scientists who received their early training at BNL or, having been trained at BNL, went on to train others in this field.

NMR Contributions to Medical Imaging (U. of IL). The Office of Basic Energy Sciences has continuously supported the research of Charlie Slichter at the Seitz Materials Research Laboratory at the University of Illinois since about 1961. There are several connections of his group to imaging and medicine. The most direct is simply that Sir Peter Mansfield, codiscoverer with Paul Lauterbur of Magnetic Resonance Imaging (MRI), was a post doc with Charlie Slichter the years 1962-1964 and has told him that he came up with the idea of imaging 10 years later while reflecting on some experiments he did while in Slichter's group. Mansfield had worked on the use of Nuclear Magnetic Resonance (NMR) double resonance to study the environment of the Cu atoms adjacent to Zn atoms in dilute Cu-Zn alloys. After leaving Slichter's group, Mansfield started working on a clever method of eliminating the broadening of the NMR lines in crystals which arises from the magnetic dipolar coupling between nuclei. Mansfield's invention of imaging arose out of thinking about what he might do if he could completely eliminate this source of broadening of the NMR lines. Thinking about the experiment he worked on while with Slichter, he suddenly realized that application of a linear magnetic field gradient would enable him to make a map of the location of the Cu atoms near to the Zn and, even more generally, that he could do with NMR something akin to x-ray diffraction for determining crystal structure. His first publication on MRI was titled *NMR Diffraction in Solids*.

Ultratrace Determination of Lead in Whole Blood (U. of FL). Professor Winefordner at the University of Florida leads research whose emphasis is upon the development of new, sensitive, selective spectroscopic methods for trace elemental analysis. His group has developed a very sensitive laser fluorescence method--electrothermal atomization laser-excited atomic fluorescence spectrometry--that has been used successfully for ultratrace determination of lead in whole blood.

Spectroscopic Technique Applied to Tumor Identification (GA Tech). Professor Mostafa El-Sayed, Georgia Institute of Technology, is supported by Basic Energy Sciences to study the proton pump mechanism of bacteriorhodopsin, a molecule that is able to convert solar energy directly into useful chemical energy. His spectroscopic expertise was called upon by colleagues in the Departments of Surgery and Pharmacy to assist in using Fourier Transform Infra-Red (FTIR) spectroscopy as a tool to identify tumors. FTIR was used to compare cultured normal and carcinogenic cells. Significant and reproducible differences in the intensities of the CH_2 and CH_3 bending modes in the normal and cancer cells were observed suggesting that changes in the

environment upon carcinogenesis induces a change in the relative absorption cross sections. The results indicate that FTIR spectroscopy may become a promising and sensitive technique for tumor identification. The work was published in the *Journal of Clinical Laser Medicine and Surgery*.

2. DISEASE TREATMENT

Drug Development:

Biotechnology Research for Increased Production of New Anticancer Drug, Taxol (ESC Agenetics Corp.). Terpenoid oils, resins and waxes from plants are important renewable resources of industrial and pharmaceutical value, and they play an essential role as natural, environmentally safe pesticides in protecting plants from insect and pathogen attack. The very large number of naturally occurring terpenoids (over 22,000 are now known) has required careful selection of model systems with which the underlying factors that control composition and yield can be understood. Research on the monoterpenoids in mint (the monoterpenoids are the simplest class of these compounds and contain only ten carbon atoms) led to the discovery of the first gene coding for the production of a monoterpenoid. The compound, limonene, has the aroma of citrus fruit and is a powerful deterrent for root and stem boring insects. The gene was patented and its use licensed to Pioneer Hybrid International for engineering into crop plants as an insect repellent. This gene is also being used by other industrial partners for the production of value added crops and ornamentals with enhanced flavor and aroma. The same approach has been applied in Pacific yew to the production of the new anticancer drug taxol that is in extremely short supply. Two of the slowest steps of the pathway were discovered, providing targets for gene isolation and the information required to more efficiently screen yew cell lines (in collaboration with the ESC Agenetics Corporation) for increased production of this valuable drug.

Mass Spectrometry (ORNL). The recently introduced Finnigan Corporation model-LCQ electrospray/ion trap mass spectrometer is selling well, especially to the pharmaceutical industry for use in drug discovery. Knowledge of early Basic Energy Sciences (BES) sponsored research at the Oak Ridge National Laboratory (ORNL) on coupling the electrospray ionization source with the ion trap configuration, along with consulting by the BES-sponsored principal investigators (Scott McLuckey and Gary Glish), played a role in stimulating Finnigan to develop this instrument. Knowledge of the BES research also apparently played a role in the development and introduction of a similar instrument (the ESQUIRE model) by the Bruker-Franzen Corporation.

Low-Temperature-Loving Microbes for Conversion of Lactose to Galactose and Glucose (Penn State U.). Basic Energy Sciences has been funding research on the biochemistry and physiology of microbes that live in "extreme" environments in order to understand the restrictions (and opportunities) of using biological systems/components in an industrial context. While much of this work has focused on hyperthermophiles (high-temperature-loving) organisms,

Dr. Jean Brenchley, Pennsylvania State University, has been examining the mechanistic and structural properties of enzymes that function at very low temperatures (from psychrophilic or low temperature loving microbes). One enzyme studied is ß-galactosidase which catalyzes the conversion of lactose to galactose and glucose. Many people are lactose intolerant and must avoid dairy products. This research has attracted considerable interest from food and dairy companies in using psychrophilic ß-galactosidases to treat dairy products to remove lactose while maintaining refrigeration.

Drugs, Neutrons, and the HFBR (BNL). The High Flux Beam Reactor (HFBR) at the Brookhaven National Laboratory is one of four neutron sources operated by Basic Energy Sciences. HFBR supports a range of neutron-based research in solid-state and nuclear physics, chemistry, and structural biology. The last research area includes studies for fundamental understand of drug interactions with the body. For drugs to be effective, it is necessary that they interact with the appropriate receptors located in the biological membrane, triggering the desired physiological response. The efficacy of a drug can be determined from a knowledge of the binding of the drug--its proper location and orientation within the receptor protein--and possibly improved by chemical modifications. For most drugs, the contrast between the drug and the membrane is so small that x-ray diffraction cannot provide this information even with a very intense beam such as provided by a synchrotron radiation source. With the help of appropriate deuterium labels, neutron diffraction can determine the location and conformation of the drug molecule in the membrane. The following are some examples of this work carried out at HFBR, where instrumentation optimized for such studies has been developed.

a. Amantadine Blocks Influenza Infections. The proteins in the coat of the virus play an important role in its replicative cycle. After the entry of the influenza virus into a target cell, one of the surface proteins of the virus, known as M2, acts as a proton channel which causes a change in the pH inside the virus. This in turn causes the virus to release its genetic material (RNA). The viral RNA uses the cell's system to replicate and to complete the cycle of infection. Neutron studies at the HFBR have shown the precise location where amantadine binds with the M2 channel and how it disrupts the multiplication of the virus by closing the channel. This work has also provided a molecular basis for an understanding of why the drug becomes ineffective when the virus mutates in such a way that the drug can no longer block the M2 proton channel. Work of this kind may help in developing even better drugs that remain effective against various mutations of the virus. The drug amantadine is sold under the trade name of Symmetrel (DuPont).

b. Citerizine, a Non-sedating Antihistamine. Unlike other commonly used antihistamines, citerizine causes minimal sedating action. Neutron diffraction studies at the HFBR have helped understand the mechanism of the drug's action and the reason for its desirable properties. By reconstituting the drug in model membranes, it was determined that the entire drug molecule is buried deep within the hydrophobic (acyl-chain) region of the membrane. Due to this strong hydrophobic binding, citerizine is unable to cross the blood-brain barrier, and hence it does not cause sedation. The drug has been approved by the FDA and will be available soon.

c. Hypertension Drugs, Propranolol and Nimodipine. These two drugs, which were studied using neutron diffraction, are given to patients suffering from hypertension. The modes of action of these drugs are somewhat different. Nimodipine is a calcium channel blocker that inhibits the flow of calcium ions across the membranes. Propranolol, on the other hand, is a compound that blocks different receptors (the so-called beta adrenergic receptors) in cellular membranes. Activation of these receptors by naturally produced compounds can result in undesirable physiological responses, such as increased cardiac rate. Propranolol has a high affinity for the binding sites of this receptor, and hence it does not allow potentially dangerous affectors to reach the beta adrenergic receptor, causing arrhythmic heartbeats and excessive dilation of the blood vessels. Neutron diffraction was used to determine the non-specific binding location of deuterated nimodipine and propanolol in model membranes. The different profiles gave the location and orientation of the drug molecules in the membranes, and thus helped in understanding their mode of action. Nimodipine is now available for treatment with the trade name Nimtop (Miles) and is given to patients with high blood pressure for improved neurological performance. Propranolol is sold under the trade name Inderal (Wyeth-Ayerst) and is given for the management of hypertension and for the long-term management of angina pectoris.

d. Tissue Plasminogen Activator--New Hope for Stroke Victims. The molecular switches and the conformational changes in enzymes caused by enzymatic regulators can be directly probed under physiological conditions by neutron scattering. An important example of such a study involves the blood clot dissolving enzyme, plasminogen, which is present in an inactive form in human blood serum and becomes activated on contact with the clot forming material, fibrin. The activation of native human plasminogen by lysine was investigated in dilute solution to gain insights into the mechanism that transforms an innocuous enzyme of the blood into an effective protease, capable of dissolving blood clots on contact. The enzyme consists of a single polypeptide chain that forms six domains, five molecular tethers called 'kringles', followed by the functional protease, or protein attacking unit. Researchers using small-angle neutron-scattering at the HFBR discovered that when a particular weak (lysine) binding site in the structure is occupied; the initially compact plasminogen structure unfolds into a string of domains and becomes active. The conformational change is reversible and the largest recorded in any enzyme. Plasmin binds to its substrate, fibrin, through kringle 1. This means that the protease at the opposite end of the flexible, open tether has a large radius within which to cleave fibrin. The determination of the enzyme activation mechanism by ligand induced unfolding was an important step leading to the genetic engineering of a new drug called tissue plasminogen activator (tPA), used to treat stroke victims. This drug, tPA, developed and marketed by Genentech, has been credited with saving the lives of about 17,000 stroke victims in the United States alone.

e. Cyclosporin Increases the Success of Organ Transplants. Human organ transplants, now nearly routine, would be impossible except for the development and use of drugs which 'trick' the immune system into accepting a foreign organ. One of the first highly successful drugs of this kind is Cyclosporin. The high-resolution neutron structure of this drug was examined using neutrons at the HFBR. The study revealed the origin of this molecule's

remarkable rigidity under a wide range of physiological (as well as non-physiological) conditions. The experimentally determined hydrogen bonding network of the fungal peptide sequence in the drug differs significantly from that previously assumed from x-ray studies, and involves a newly found and tightly bound water molecule. The hydrogen network is closed within the structure and leaves no polar anchors for interaction with solvents. These qualities make the drug hydrophobic and impart a remarkable structural stability. Both features contribute to the successful use of Cyclosporin to inhibit the activation of populations of cells that cause rejection of the organ transplants by the immune system. The closed and rigid structure of Cyclosporin provides a valuable further clue in the continuing search for better immuno-suppressive drugs.

f. Pain Reliever for Bone Cancer. The HFBR produces ^{117m}Sn for the radiopharmaceutical Sn-DTPA (dietheylene triamine pentaacetic acid) which is being tested as a bone seeking pain reliever for the 320,000 new cases of bone cancer reported in the United States every year. Unlike narcotic drugs, it does not sedate the patient, while providing a selective radiation dose to the bone tumor and but little to the bone marrow. Thus, it does not interfere with the marrow's ability to fight infection or with the bloods ability to clot. Also, for experimental cancer research, the HFBR produces ^{199}Au which is incorporated into a drug containing 11 Au atoms attached to an anti-tumor antibody which binds to the tumor cells. ^{199}Au is lethal for a radius of 10 cells and has a short half-life, 3.1 days, which makes it ideal for this purpose.

The Effects of Drugs on Osteoporosis (ORNL/Harvard U.). Oak Ridge National Laboratory (ORNL) researchers have done neutron scattering work on the structure of bone. Published studies involve how the mineral forms in bone and addresses the problem of osteoporosis and loss of mineral in response to certain drugs. Of interest is whether certain drugs can induce osteoporotic-like conditions. An example is the fluoride added to drinking water which was suggested to cause loss of mineral in bone. ORNL researchers found this not to be true at low doses. However, hydrocortisone has an effect at all doses. This work was done in conjunction with Harvard University.

Rational Drug Design (ANL-APS/IMCA). The Advanced Photon Source (APS) at the Argonne National Laboratory is one of four synchrotron light sources operated by the Office of Basic Energy Sciences. The brilliant X-ray beams generated at APS are used by scientists to probe material structure in greater detail than ever before, opening new vistas of research in materials science, chemistry, physics, biotechnology, medicine, and the environmental, geological, agricultural, and planetary sciences. One such consortium of scientists, from a dozen major pharmaceutical research laboratories, is using advanced x-ray crystallographic techniques at the APS to determine the structures of biological macromolecules. This consortium is the Industrial Macromolecular Crystallography Association (IMCA), whose member companies are Abbott Laboratories, Bayer, Inc., Bristol-Myers Squibb, GlaxoWellcome Inc., Eli Lilly & Company, Merck & Company, Monsanto Company Inc., Parke-Davis Pharmaceutical Research, Pharmacia & Upjohn, Inc., The Procter & Gamble Distributing Company, Schering-Plough Pharmaceutical Research, and SmithKline Beecham Pharmaceuticals. Understanding these biological structures

serves to guide protein engineering efforts to develop new molecules with specifically improved pharmaceutical properties. Proprietary research being carried out by these investigators is contributing directly to the rational design of biologically active compounds for medical use. The pharmaceutical companies active in this consortium anticipate that research being carried out at the APS will significantly lower the costs and shorten the lead time required to introduce new drugs and consumer health products into the world marketplace.

Chiral Drug Separations (LSU). Studies on chiral separations using chiral calixarene chemistry by Professor Warner at Louisiana State University may have a number of applications in the pharmaceutical industry, e.g., for the separation of chiral drugs. Professor Warner's team was the first to achieve such separations using this form of host-guest chemistry.

Analytical Detectors for Medical Technologies (Ames). Professor Ed Young at Ames Laboratory has led the development of an optical rotation detector for chromatography. This device received an R&D 100 Award. There are four pharmaceutical companies that have constructed such instrumentation in collaboration with the researchers. In addition, the Ames researchers developed a fluorescence detector for capillary electrophoresis which also received an R&D 100 Award. Two pharmaceutical companies and two cancer research laboratories have constructed such instrumentation in collaboration with the researchers.

Preparative Chromatography For Commercial Medical Applications (U. of TN). The Basic Energy Sciences funded work by Professor George Guiochon at the University of Tennessee on the chemical and physical properties of large size chromatography columns has provided the basis for developing these separation systems into a commercial reality. Chromatographic separations are achieved by virtue of the differences in the interaction between components of a liquid and the surfaces which make up the column--the stronger the interaction the slower the passage of that component along the column. The large surface area required is obtained by packing small particles into the column. Professor Guiochon found that the uniformity of flow throughout the cross section on a large column was strongly dependent on residual stresses which resulted from the packing process. Packing procedures which reduce residual stress yield practical columns with greatly improved performance. These columns are especially important for their ability to separate enantiomers. The two enantiomers of a pharmaceutical are considered by the FDA as two different chemicals and must be validated separately. Often, one is much more active or safer than the other. Depending on the product, the enantiomeric separation is done at different stages of the synthesis. Sometimes two such separations are done, one early to work mainly on an enatiomer, the other at the end to eliminate small amounts of the other enantiomer formed at one step or the other. This type of chromatography is used for the selective extraction of specific proteins from fermentation broths and for their purification. For example, Ely Lilly uses three successive steps of preparative chromatography to purify recombinant human insulin, at the scale of 15 tons/year. The method is also used to purify critical intermediate in the synthesis of pharmaceuticals.

Photochemical Studies on the Light-Activated Drug Hypericin (BNL). The herbal remedy, St. John's Wort, contains the compound hypericin. When hypericin is exposed to light, it is toxic to

tumors and HIV, the human AIDS virus. The efficacy of antiviral hypericin is being evaluated presently in clinical trials. In collaborative studies involving Dr. Edward Castner, Brookhaven National Laboratory (BNL), and Dr. Jacob Petrich, Iowa State University, the fundamental mechanistic photochemistry of hypericin has been elucidated. Fluorescence upconversion, a novel laser spectroscopic technique available at BNL, was used in showing definitively that the primary photochemical process, upon interaction with light, is excited-state intramolecular proton or hydrogen atom transfer. Any incomplete proton or hydrogen atom transfers would acidify the aqueous solution immediately surrounding hypericin, which may be of importance in its toxicity to viruses.

Radiation Therapy:

HFIR-produced Medical Isotopes (ORNL). The High Flux Isotope Reactor (HFIR) at the Oak Ridge National Laboratory (ORNL) is one of four neutron sources operated by the Office of Basic Energy Sciences (BES). HFIR produces the Nation's most intense continuous beam of neutrons for materials research and isotope production. Several companies have based the commercialization of new medical products on the availability of therapeutic medical radioisotopes from Office of Biological and Environmental Research (OBER) supported research at HFIR. These include tungsten-188 for tungsten-188/rhenium-188 generators, tin-117m and rhenium-186. For example, Mallinckrodt Medical Inc. has licensed an invention from ORNL that could save more than 100,000 people from having additional heart surgery. Of the 400,000 balloon angioplasty procedures that are performed annually in the United States, 30 percent of patients require additional surgery because their arteries clog up again. Although a variety of pharmacological approaches are being explored to inhibit restenosis after balloon angioplasty, ionizing radiation has been found to be one of a few non-surgical and easily performed procedures which is effective. The use of the special radioactive isotope--rhenium-188--prevents this arterial blockage from forming and thus will help save time, lives and costs. A highly concentrated form of rhenium is needed for this type of procedure and is provided by a special ion exchange system. The technology was developed at ORNL by Russ Knapp, Arnold Beets, Saed Mirzadehand, and Stefan Guhke. The clinical application of rhenium-188 for coronary artery irradiation has been approved by the U.S. Food and Drug Administration for use at Columbia University Medical Center in New York. Besides treating restenosis, rhenium-188 is used in physician-approved trials in collaboration with ORNL for the treatment of bone pain from skeletal metastases of cancers of the breast, lung, and prostate.

HFIR generated medical radioisotopes are also used to treat forms of cancer that do not respond well to conventional radiation treatments. For some of these, the unusual radiations from ^{252}Cf have a much better success. As part of the National Transplutonium Element Program sponsored by BES, Californium-252 is produced by irradiating capsules containing plutonium, americium, and curium in the HFIR and then separating the ^{252}Cf. More than 650 patients were treated with ^{252}Cf at the University of Kentucky Medical Center for cancer of the cervix and other types of cancer. Virtually all received a decided improvement in the quality of life and many received improved life expectancies. Those who received treatment before their cancers were advanced were cured. Over 1,000 patients in Russia and Lithuania have also been treated with good

success by their medical establishments. In the United States, the main thrust for the last two years has been at Wayne State University, in Detroit. There, at the Gershenson Radiation Oncology Center, Drs. James Fontanesi and Paul J. Chuba have treated 30 patients with tumors being of both high and low grade sarcomas. Their major difficulties lay in the relatively long treatment times (patient discomfort) and excessive exposure to the staff. To accommodate their needs, ORNL is in the process of developing more intense, but physically smaller sources that can be manipulated by after-loading machines like the ones that are routinely used to handle gamma-emitting brachytherapy sources. (Brachytherapy is the process wherein the radiation source is placed directly in contact with or even within the cancerous tissue.) If successful, ^{252}Cf may be put through Phase II and Phase III clinical trials and perhaps become standard therapy for certain forms of cancer.

Reaction Mechanisms Important for Cancer Radiation Treatment (ANL). Radiation has been a common treatment for cancerous tumors. The radiation sources most commonly used for medical treatment are X-ray or gamma sources and high-energy electron sources. These are all classified as low linear energy transfer (low-LET) radiation. Cells containing oxygen are much more sensitive to low-LET radiation sources; unfortunately, because of the lack of blood flow, cancerous cells have little oxygen in them. Radiation sources such as cyclotrons, synchrotrons, neutrons, and other high-LET radiation sources have also been explored for cancer treatment because all cells, both with and without oxygen, are almost equally sensitive to such radiation. Because the chemical species created by both high-LET and low-LET radiation are the same, it must be the differing nonhomogeneous reactions in the two radiation fields that determine the differences. To understand the origin of these effects so that one might potentially exploit the differences, considerable effort was expended in creating computational models. However, there were no experimental data available in the time domain that would allow testing of the computational models. With the support of BES, the Chemistry Division at Argonne National Laboratory measured the kinetics of the two primary radicals created by ionizing radiation--the hydrated electron and the OH radical. The experimental results showed that the theoretical rates predicted for nonhomogeneous reactions were incorrect by an order of magnitude. Since that time, these results have been used by all theoretical simulations as a test of the calculational models. These data are still the only generally accepted data for the nonhomogeneous reaction in water. With the updated models, predictions about how best to treat cancers using radiation have been made as well as models of the mechanism of radiation therapy and radiation damage.

Production of ^{212}Bi for Medical Therapy (U. of Chicago/ANL). The use of alpha emitters for medical therapy is being actively investigated by physicians and scientists at the University of Chicago Lying-In Hospital, Department of Obstetrics and Gynecology. The alpha-emitting radionuclide ^{212}Bi (60.6 min half-life) has been found to be very effective for intraperitoneal therapy against microscopic ovarian carcinoma in animals. Plans are underway to extend the treatment to human patients. One of the most stringent requirements for radionuclides used for therapeutic purposes is the complete absence of long half-life parents. If the ^{212}Bi used to treat a patient does not decay to background levels because of the presence of even traces of much longer half-life parents, such as ^{212}Pb or ^{224}Ra, the patient may be exposed to a higher level of radiation than is desirable. The most critical step in the production and decay scheme of ^{212}Bi is

the isolation of ^{212}Pb (the parent of ^{212}Bi) from the last traces of ^{224}Ra and ^{228}Th. Members of the Chemical Separations Group of the Chemistry Division at Argonne National Laboratory have developed a novel extraction chromatographic resin that is highly selective for Pb^{++}, and to a much lesser extent Sr^{++}, over all other metal ions in the periodic table. The Pb/Sr selective resin uses the principle of metal ion recognition. A macrocyclic polyether molecule has been designed to selectively bond to Pb^{++} in the presence of high concentrations of its parents, ^{224}Ra, ^{228}Th, and ^{232}U. The ^{212}Bi solution resulting from this production process is finally neutralized with NaOH to produce a saline solution to be administered to a patient by intraperitoneal injection.

Surgery:

Excimer Laser Energy for Angioplasty, Angina Pectoris, and Retinopathy (ANL). Drs. Dieter Gruen, Michael Pellin, and Charles Young, at the Argonne National Laboratory, had been working on achieving a fundamental understanding of the sputtering process since 1979. They used laser fluorescence spectroscopy to measure the velocity distribution and occupation of low-lying electronic sites of sputtered atoms and in 1982 developed an argon-ion-pumped, ring dye laser system amplified with a 308 nm XeCl excimer laser. To achieve efficient amplification of the dye laser, the excimer beam properties had to be closely controlled, and the investigators quickly discovered that at the focal point of the excimer, a very unusual phenomenon occurred. The IBM paper punch cards routinely used to locate the laser focus were perforated by the laser beam after a few pulses, leaving clean, tiny holes, but without the char marks always seen when the same procedure was used with other lasers. The phenomenon had all of the earmarks of "ablative photodecompositon," suggesting that a multiphoton mechanism was at work to remove or ablate material from the surface without causing extensive thermal damage to the surroundings.

Subsequently, in 1983, these researchers were consulted about a long-standing problem in the field of laser angioplasty. Cardiovascular disease, in particular the formation of plaques in arteries leading to the heart, is one of the most serious public health problems, not only in the United States but throughout the world. Heart attacks and morbidity frequently occur if such a condition is left untreated. By-pass, or open heart surgery, was the only treatment for this condition until various angioplasty procedures were developed. One frequently used procedure is balloon angioplasty; however, this procedure has been found to have restenosis (reblockage) rates of 30-40 percent, requiring the procedure to be repeated. Keeping in mind their IBM punch card experience, the researchers believed the use of energy from their excimer laser, conducted through a fiber-optic catheter, might allow plaque removal by the above "evaporative" or ablative process. Approaches using other types of lasers were unacceptable due to accompanying effects to surrounding tissue in the form of thermal and acoustic damage (charring). In order to be effective in laser angioplasty, the laser energy needed to be transmitted by a fiber-optic catheter from its origin at the excimer laser to the locus of the arterial obstruction, namely the plaque inside the artery. This distance can be several meters, and therefore the cross section for absorption by the fiber-optic must be very low. Fortunately, the researchers also had extensive related laser/fiber optic application expertise for the measurement of metal impurities within plasmas of fusion energy machines called Tokamaks. This experience

with optical fibers had showed that transmission of light at wavelengths below about 280 nm is not feasible; but at 300 nm and above, high purity silica fibers are more than 95 percent transmissive. Their inventive ideas were reduced to practice at Argonne in June 1983 when a plaqued cadaveric piece of artery was exposed to the focused 308 nm excimer radiation. The disease tissue was cleanly removed, and it was obvious by visual inspection and subsequently confirmed by detail histologic examination that surrounding tissue did not suffer charring or burning. Subsequent, successful commercialization of 308 nm excimer medical lasers and fiber-optic catheter delivery systems has been accomplished by the Spectranetics Corporation of Colorado Springs, Colorado. The worldwide sales of this company are approaching $30M. Today, the 308 nm excimer laser is used to remove certain kinds of arterial lesions, to clear stents in restenaosis occurrences, to clear totally obstructed saphenous vein by-pass grafts, to remove blockages in the peripheral vascular system leading to intermittent claudication, and as an extremely effective means to remove incarcerated implanted pacemaker and defibrillator leads. Very encouraging results are also being obtained with percutaneous myocardial revascularization (PMR). In this procedure, a catheter is placed inside the ventricle, and about 30 small holes are produced by the laser/fiber optics system in the myocardium by ablation. Healing takes place rather quickly, but the myocardium is stimulated to angiogenesis. The small blood vessels produced during this process improve oxygenation of the heart muscle, thus decreasing symptoms of angina pectoris.

Another, potential application of this technology is as an intraocular surgical tool to remedy in certain diabetic patients a condition known as proliferative retinopathy. In these patients, structures grow from the retina into the vitreous humor of the eye, which are referred to as "bands." With patient aging, the vitreous humor shrinks, and because the bands are attached to the retina, retina detachment occurs. In order for the retina to become reattached, a lengthy surgical procedure is required in which micro scissors are used to cut the bands. The Argonne research team, working with ophthalmological surgeons at the Eye Institute of the Medical College of Wisconsin in Milwaukee, demonstrated very successful protocols in clinical experiments on the eyes of rabbits. The laser energy delivered by the laser/fiber-optic cable system to the site of the bands in the vitreous humor cut the bands akin to the cutting action of traditional micro scissors. Excellent control over the cutting action of the laser was achieved. The President of the American Ophthalmological Society has commented favorably on the pioneering aspects of this work in an editorial in *The American Journal of Ophthalmology*.

Ion Beam Processing of Wear Surfaces in Artificial Prostheses (U. of AL/ORNL). This technology, an outgrowth of BES research in collaboration with the University of Alabama at Birmingham in the 1980s, greatly enhances the corrosive wear resistance of artificial prostheses such as hip, knee, and finger implants. It was originally developed for a surgical titanium alloy used primarily for artificial hip and knee joints. This alloy has excellent biocompatibility but poor wear resistance in body fluids. The ion implantation process increases the wear resistance of the alloy by a factor of 1000, eliminating corrosive wear of the metal as an issue. More than 500,000 Americans have received ion-implanted titanium hips and knees over the past decade. Recently, the FDA issued a ruling requiring that all wear applications of titanium in the body undergo the ORNL treatment (which is now provided by Spire and other commercial ion

implantation services). In addition, ion implantation is also being used to toughen the wear surfaces of other prosthetic materials such as chrome-molybdenum alloys.

Biomimetic Coatings for Bone Implants (PNNL/ABCI/Implant Innovations, Inc.). Each year, almost 500,000 patients receive hip or knee implants worldwide, and an additional 500,000 require reconstruction of bone injuries resulting from congenital birth defects and athletic injuries. While current implants can allow people to live more active and normal lives, there are often problems associated with inadequate anchoring of the metal prosthetic devices. Either due to poor bone growth or rejection, small gaps between natural bone and the implant increase over time resulting in implant failure, which then requires additional surgery for removal and replacement of the implant. Research at the Pacific Northwest National Laboratory (PNNL) supported by the Office of Basic Energy Sciences has led to the development of a variety of processing routes to ceramic thin films and composites. Under subsequent CRADAs funded by the Office of Energy Research, PNNL researchers have teamed with Applied Biological Coatings and Implants (ABCI) of Dallas, Texas, and Implant Innovations, Inc., of Bell Garden, Florida, for development of bioactive calcium phosphate coatings for orthopedic implants. The low temperature processes allow both highly porous and smooth implant surfaces to be coated without affecting surface texture or clogging implant porosity. In addition, the processes are mild enough to allow the incorporation of biomolecules, such as growth factors and antirejection agents, to be incorporated into the surface coatings.

Interfacial Engineering (RPI). The research (generally termed "interfacial engineering") of Professor George Belfort at Rensselaer Polytechnic Institute is geared toward surface modification of polymer films/membranes and methods to reduce membrane fouling--both of importance for chemical separations and filtration. This research is directed at measuring the intermolecular forces between proteins and polymeric surfaces, and also the modification of these interactions by reducing the attractive forces between the proteins and the polymeric films. The fundamental knowledge obtained also could be of direct relevance to interfacial interactions at the surfaces of biomedical devices such as drug delivery components and catheters. Protection of interfaces such as teeth could also benefit from this work.

3. DISEASE UNDERSTANDING

Osteoporosis (LLNL-SSRL/U. of CA). Osteoporosis, a disease of excessive skeletal fragility, is a significant public health problem that affects more than 30 percent of women over the age of 65 years. It is known that estrogen deficiency at menopause results in accelerated bone remodeling with loss of trabecular bone and increased bone fragility. Researchers lead by John Kinney at Lawrence Livermore National Laboratory (LLNL), in collaboration with scientists at the University of California at San Francisco, have been developing x-ray tomography using synchrotron radiation at the Basic Energy Sciences' Stanford Synchrotron Radiation Laboratory (SSRL) to investigate osteoporosis. Under LLNL and NIH funding, initial research has studied the time sequence of changes to trabecular bone structure with estrogen loss in the ovariectomized rat model of osteoporosis. Using a new, high resolution imaging detector, the

researchers have visualized the architectural changes that occur with estrogen loss, and have demonstrated that irreversible architectural changes occur within five days post ovariectomy. Furthermore, it has been established that estrogen replacement therapy, though stabilizing bone mass and trabecular architecture, does not recover trabecular connections once they are lost. This is important because the researchers have more recently determined that recovery of trabecular connectivity is essential for recovering strength. Thus, the research strongly supports early intervention in treating osteoporosis with hormone replacement or antiresorptive compounds such as bisphosphonates.

The scope of this research is now being expanded by studying how a new class of anabolic agents that increase bone mass affect trabecular bone architecture and strength. Studies of parathyroid hormone fragment hPTH(1-34) in clinical trials of osteoporotic women, for example, have detected increased bone mass in the lumbar spine and hip, and a reduction in the number of new or incident vertebral fractures. This suggests that PTH increases the strength of the trabecular bone by increasing bone mass and by changing trabecular bone architecture. A new focus includes seeking to develop a better understanding of how bone building agents work, and when they need to be administered to prevent bone fragility.

Diffraction Enhanced Imaging Applied to Mammography (BNL/IIT/U. of NC/NC State U./ANL-APS). A project team including researchers from Brookhaven National Laboratory (BNL), Illinois Institute of Technology, University of North Carolina Department of Radiology, and North Carolina State University has invented a new imaging technology called Diffraction Enhanced Imaging (DEI). This technique is producing images of malignant tissues in which the detail, resolution, and visibility of tumor far exceeds that of conventional imaging. In addition, the technique is sensitive to both diffraction and refraction in the tissues, information not available in standard technology. The team is using beamlines at the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory and the Advanced Photon Source (APS) at Argonne National Laboratory for development of this technology.

Laser-Based Analysis of the Tracer Isotope Ca-41 (PNNL). Researchers at the Pacific Northwest National Laboratory (PNNL), in collaboration with a group at the University of Mainz in Germany, are developing a new diode-laser-based method for ultra trace analysis of the radioisotope Ca-41. This isotope, which is very long lived (half life of 104,000 years) decays via electron capture and thus has a very low radiation hazard when used as a tracer in medical-related studies such as the biokinetics of calcium exchange in human osteoporosis. Currently, accelerator mass spectrometry (AMS), which can be performed at only a few large facilities around the world, is the only method with sufficient sensitivity and selectivity for these measurements. The laser-based method should provide a relatively low-cost, bench-scale instrument with detection capabilities similar to AMS, but with faster measurement times.

Structure of the Putative Human Tumor Suppressor Protein (ANL-APS/Columbia U.). Numerous research groups, or Collaborative Access Teams (CATs) as they are called at the Argonne National Laboratory's Advanced Photon Source (APS), comprised of members from universities, national laboratories, and industry, are applying new and advanced instrumentation

and techniques to determine the detailed molecular structure of highly complex biochemicals ranging from proteins, enzymes, and genetic substances to complex virus systems. The high brilliance x-ray beams at the APS permit much greater structural information to be extracted and from smaller biomolecular crystallites that take less time to produce in the laboratory. The net result is an acceleration of information acquisition about these biomolecular structures, how they fold together and bind to other biological components, and ultimately how they function physiologically. This research holds real promise for new insights into human biochemistry and physiological function, disease, and genetic function. These are all key areas of understanding needed for guiding future medical advances, including drug design and development. For example, research at the Structural Biology Center (SBC) at the APS by C. D. Lima and W. A. Hendrickson, College of Physicians & Surgeons of Columbia University, has determined the structure of the putative tumor suppressive fragile histidine triad (FHIT) protein to 1.9-Angstrom resolution. The researchers, funded in part by a Helen Hay Whitney Foundation Fellowship and by a NIH grant, employed the multiwavelength anomalous diffraction (MAD) technique that is enhanced by the high-brilliance x-ray beams produced at the APS. The MAD data was used to obtain the detailed structure for both the free and ligand-bound forms of the FHIT. FHIT, a member of the histidine triad family of proteins, derives from a fragile site on human chromosome 3 that is commonly disrupted in association with human cancers, although definitive evidence supporting its role as a tumor suppressor has yet to be elucidated. Structural and biochemical analyses of these diverse human HIT members should better focus the search for the *in vivo* function of HIT proteins and potential applications in medicine, in particular for treatments of cancers.

Structure of Intermediates in Chemical Carcinogenesis (Purdue U./OR State U.). Research on ion trap mass spectrometry by Professor Graham Cooks at Purdue University has resulted in structure determinations of DNA adducts with syn- and anti-dibenzo[a,l] pyrene diol epoxides. The nucleoside adducts were structurally characterized using ion trap tandem mass spectrometry. Dibenzopyrene diol epoxides are key intermediates in chemical carcinogenesis, and the structures of their adducts with DNA constituents are essential information needed to develop strategies to reverse the binding. The study uses modern ion trap methods for the difficult task of distinguishing closely related structural isomers. The work is being done jointly with Prof. William E. Baird, Director of the Cancer Center, Oregon State University. Basic Energy Sciences funds efforts at understanding and controlling ion motion in the ion trap to continue to improve performance of this type of mass spectrometer, including (a) the first MS/MS ion trap experiments, (b) the first broad-band non-destructive detection experiments, (c) the recently introduced cylindrical ion trap, and (d) ongoing simulations of ion motion in ion traps. As a result of the Purdue work and that of other BES-funded groups, a number of laboratories are using ion trap mass spectrometry to determine the structures of peptides and proteins, nucleotides and glycoproteins.

Protein Crystallography and Biomicroscopy at the Advanced Light Source (LBNL-ALS). The Advanced Light Source (ALS) at the Lawrence Berkeley National Laboratory is operated by the Office of Basic Energy Sciences and is a third-generation synchrotron radiation user facility of very high brightness optimized for the ultraviolet and soft x-ray regions. High brightness

translates into high spatial resolution at the sample. In the area of life science, the ALS offers a very competitive protein crystallography facility as well as programs in biomicroscopy. X-ray crystallography is the primary method used to determine the atomic structure of biological macromolecules--proteins, viruses, DNA and RNA. This structural information has led to an immense increase in our understanding of the biological processes that are mediated by these molecules. Moreover, specific therapeutic agents for human disease can now be designed based on the structure of the molecules involved--a significantly more rapid route to drug discovery than previously available. The undeniable success of protein crystallography is increasing the demand for more facilities. Furthermore, the Human Genome Project will identify sequences for a large number of proteins for which structural information will need to be obtained, and the number of new sequences is growing substantially faster than that of new structures. To help meet these challenges, the ALS Macromolecular Crystallography Facility (MCF) is now in operation. The MCF is based on a beamline funded by the Department of Energy's Office of Biological and Environmental Research through LBNL's Physical Biosciences Division. In addition to conventional monochromatic crystallography, the beamline is optimized for multiple-wavelength anomalous diffraction (MAD) experiments. MAD methods allow for the determination of the phases, in addition to the amplitudes, of diffracted beams; both are required for Fourier synthesis of the electron density map that represents the molecular structure. In all, more than 60 users from 18 different groups collected data at the MCF during the period from November 15, 1997 to January 31, 1998. MAD data include ApoE, a protein important in cholesterol metabolism, by Lawrence Livermore National Laboratory (LLNL), to 1.8 Å, and MJ0577, a hypothetical protein from the genome of a hyperthermophile, to 1.6 Å, by researchers in LBNL's Physical Biosciences Division. The ApoE structure was recently determined with these data by Bernhard Rupp's group at LLNL. The MJ0577 structure can act as a model for the use of biomolecular structure as an approach to understanding the function of sequences from the Human Genome Program. A MAD data set from HIV integrase was collected to 1.8 Å resolution by the group of Senyon Choe at the Salk Institute in less than 10 hours in order to illuminate the role of this protein in AIDS and for the design of improved therapeutic agents.

Data from several microcrystals were also collected. In the most extreme case, scientists from Roche Biosciences collected an entire data set from a frozen microcrystal of collagenase with an inhibitor with dimensions of ~ 40 x 30 x 5 microns to 1.8 Å resolution and have completed the structure determination. They have also completed the structures of two other collagenase/ inhibitor complexes from microcrystals. These protein/inhibitor complexes are a part of their structure-based drug design program.

Researchers interested in eucaryotic transcription factors have been able to collect data from crystals which are intractable without the bright x-rays from the MCF. Robert Tjian's group at the University of California-Berkeley, have collected data from one transcription factor to 2.9 Å, and Mike Botchan's group (UC-Berkeley) collected data from the E2 activation domain to 2.2 Å. These protein structures will significantly enhance our understanding of the process of eucaryotic transcription.

One of the most challenging projects is the determination of the structure of the ribosome which consists of 52 proteins and 3 RNA molecules. Researchers from Harry Noller's group at UC-Santa Cruz have been able to make significant progress in improving the resolution of diffraction data from ribosome crystals. Currently data sets extending to 10-12 Å have been obtained.

"Smart Materials" for Selective Detection of Small Molecules--Glucose (LBNL). Under support from the Office of Basic Energy Sciences, a research group under the direction of Raymond Stevens and Quan Cheng at the Lawrence Berkeley National Laboratory (LBNL) made a major advance in the development of colorimetric biosensors--sensors that quickly change color in response to the presence of a defined target. The biosensors exploit a property of a class of "smart materials" known as "induced fit" enzymes. These are biological polymer catalysts which change their shape upon binding to specific molecules. Based on biosensors previously developed by Deborah Charych and coworkers at LBNL, Stevens constructed a new biosensor with the induced fit enzyme hexokinase attached at many points to a polymerized film of diacetylene monomers (PDA). (Hexokinase is an enzyme that aids in the metabolism of the sugar glucose by binding and chemically modifying it.) Exposing the sensor to dilute solutions containing glucose produced the characteristic blue to red color change of the sensor, demonstrating that the shape change in hexokinase produced by the binding of glucose to it produce sufficient mechanical stress in the PDA film to change its color. This work demonstrates the first use of "smart" proteins to activate a colorimetric solid state sensor. Although a number of more sensitive sensors for glucose are available, most are far more complex to use. This "proof of principle" opens the door to the development of colorimetric sensors for a wide variety of small molecules not now easily detected.

DNA Sequencing Instrument (SNL-CRF). Research at the Combustion Research Facility (CRF) of Sandia National Laboratories, Livermore, has resulted in the commercial development of a DNA sequencing instrument. The instrument is based upon capillary gel electrophoresis separation with ultra-sensitive laser-induced fluorescence detection. The work is the result of a CRADA with Beckman Instruments whose goal was to develop chemical/biochemical sensor technology that coupled advances in solid-state laser and micro-separation techniques. It addressed limitations restricting the use of powerful micro-separation techniques to the research laboratory due to the high demands placed upon the detectors to measure the small sample volumes. The lasers used in the new instrument are diode based with emissions in the near-infrared. The CRF played a major role in the design of the optics and the detection electronics. Halfway through the three-year CRADA, Beckman decided to move forward with full development and production of the instrument.

Droplet Dispensing for Clinical Diagnosis (Purdue U.). The use of electric fields offers a potentially powerful means for enhancing the efficiency of and reducing waste generation in various unit operations that are used in the separation and purification of chemicals. A central issue in electro-separations is the dispersion of one phase into another phase, which simply entails the creation of drops of the first phase in an otherwise continuous second phase. During the past decade, Basic Energy Sciences has supported the research of Professor Osman Basaran at Purdue University for a fundamental understanding of the factors that control the creation of

satellite droplets during drop formation. As it turns out, this ability to create and handle single micro droplets is also important for applications in clinical diagnostics and genetics research. This includes being able to dose various liquids in precise amounts and to place them exactly where they are needed. Packard Instrument Company, a manufacturer of such drop dispensing instruments, has consulted with Professor Basaran to better understand and improve its drop production techniques.

Potential New Diagnosis Procedures for Alkaline Phosphatases (VA Commonwealth U.). Work by Professor Rutan at Virginia Commonwealth University on kinetic detection, subsequent to electrophoretic or thin-layer chromatographic separations, coupled to third-order data analysis methods has medical implications. It is anticipated that the research will lead to new diagnostic procedures for difficult to separate isoenzyme systems, such as alkaline phosphatases.

Secondary Ion Mass Spectrometry Imaging of Biological Tissues (ORNL/NWU/NIMH/NIAAA/ Johnson & Johnson). For years, researchers at Oak Ridge National Laboratory have been trying to apply secondary ion mass spectrometry (SIMS) to the analysis of biologic tissue--a very challenging application. There are plenty of barriers to overcome. However, these researchers have succeeded in part and are collaborating with researchers at Northwestern University and the National Institute of Mental Health, National Institute on Alcohol Abuse and Alcoholism, and have a exploratory project with Johnson & Johnson regarding contact lenses. These projects include mapping the distribution of gadolinium compounds in the vicinity of cardiac infarcts, the distribution of phospholipids during development and as a consequence of alcoholism, and the protein buildup on contact lenses. They also have a proposal pending to develop a monitor of calcium deposition in bone. BES supported the development of this critical technology of large field of view secondary ion imaging, cluster ion impact, and bromobenzene and fullerene primary ion beams.

Mass Spectrometry for Lipoproteins Studies (TX A&M U.). Mass spectrometry is central to research in areas such as protein chemistry, DNA chemistry, genomics, and proteomics. The implementation of mass spectrometry in virtually every biochemistry and molecular biology laboratory around the world can be traced to a few laboratories working in fundamental areas of instrumentation development and ion chemistry. Mass spectrometry methods such as electrospray ionization (ESI), matrix-assisted laser desorption ionization (MALDI), collision-induced dissociation (CID), and their subsequent instrumentation developments, are all outgrowths of research in experimental physical chemistry. Virtually all the medical related research that involves mass spectrometry is now based on ESI and MALDI and time-of-flight (TOF) mass spectrometers. Basic Energy Sciences supports research involving the development and use of ESI, MALDI, and TOF mass spectrometers. The research is aimed at understanding the basic processes involved in ESI and MALDI as well as the implementation and development of more sensitive, high performance TOF instrumentation and mass spectrometry based methods. Texas A&M University researchers have used the mass spectrometers provided by Basic Energy Sciences projects to study human lipoproteins, especially HDL. They have used high resolution TOF-MS combined with MALDI to profile HDL in the molecular weight range 6-30 kda in human subjects. Over 30 apo isoforms have been detected in the HDL fraction by the

MALDI/TOF technique, whereas only 11 are detected by ESI-TOF MS. The researchers also detected and identified several new apolipoproteins. HDLs remove excess cholesterol from muscles and arteries and deliver it to the liver for excretion. Low concentrations of HDL increase the risk of coronary artery disease.

DNA Separation and Analysis (U. of DE). An important spin-off of the work by Professor Wirth at the University of Delaware on polymer encapsulation of silica is a significant improvement of capillary electrophoresis for the analysis of proteins. Protein analysis is vitally important in medical research and clinical analysis, and capillary electrophoresis has opened the door to fast analysis; however, its practice has been undermined by surface instability. Professor Wirth's successful implementation of surface-confined polymerization allows controlled formation of cross-linked polymer films for the first time. This cross-linking provides the level of stability needed for practical use of capillary electrophoresis in clinical analysis and medical research.

Biological X-Ray Microscopy (LBNL-ALS). Diffraction limits the spatial resolution of visible-light microscopes to distances of about one-half the wavelength of the light used; as a result, features in biological systems such as subcellular structures smaller than a few tenths of a micrometer are not clearly imaged. Residing near the short-wavelength end of the electromagnetic spectrum, the x-ray microscope offers a way to significantly improve the resolution. Moreover, because it obtains image contrast by means of x-ray absorption by specific elements (determined by the x-ray wavelength), the x-ray image contains features that do not show up in optical micrographs, which are not element-specific. Although the spatial resolution of an x-ray microscope cannot match that of an electron microscope, which will remain the instrument of choice for imaging the smallest structures, the ease of sample preparation (a few minutes in the investigator's laboratory) and the ability to direct the imaging process at the beamline make x-ray microscopy a user friendly technique with the ability to deliver results rapidly, whereas electron microscopy requires that the specimen be sectioned into thin slices and subjected to other sample-preparation procedures (dehydrating, fixing, staining, etc.) that can alter the structure. Preserving the specimen in its natural form also means that spatial relationships between the structures and their environments can be discerned.

Designed, built, and operated by the LBNL Center for X-Ray Optics, the high-resolution zone-plate x-ray microscope XM-1 is a direct-imaging instrument on a bend-magnet beamline at the Advanced Light Source (ALS) operated by the Office of Basic Energy Sciences. The microscope is optimized for experiments at wavelengths from 2 nm to 5 nm (photon energies from 250 eV to 600 eV). This wavelength range covers the so-called water window below the K absorption edge for oxygen (2.3 nm) but above that for carbon (4.3 nm) so that water is transparent but cellular structures containing carbon are not. In the water window, imaging biological structures in aqueous, near-natural environments is possible. The microscope includes methods for precise control of sample position and fully integrates two state-of-the-art visible-light microscopes that permit examination of the sample before and after x-ray imaging and that permit the researcher to study samples that require phase-contrast or visible-light fluorescence for location of significant features.

Researchers Carolyn Larabell, Sophie Lelievre, Donna Hamamoto, Mina Bissell and Werner Meyer-Ilse at LBNL recently developed the methodology to label proteins in hydrated cells for examination in the x-ray microscope (XM-1). Using this approach, they have obtained images at higher spatial resolution (40 nm to 50 nm) than light microscopy and can examine samples 10 μm thick in a liquid environment at atmospheric pressure. To label proteins, they used a commercially available probe, FluoroNanogold™ (Nanoprobes, Inc., Stony Brook, NY), which has both fluorescein (FITC) for visualization in the light microscope and 1.4 nm Nanogold™ particles which readily enter permeabilized cells. The gold particles can subsequently be enhanced with silver to create aggregates visible in the x-ray microscope. The researchers have used this technique to label proteins in the cytoplasm and nuclei of human mammary epithelial cells.

It is important to point out that immunolocalization analyses in the x-ray microscope also reveal information about the underlying structural components of the nuclei. Although the skeletal components of the cytoplasm, referred to as cytoskeleton, have been well characterized, comparable nuclear structures remain controversial. The controversy results from the fact that examples of nuclear structure are based on electron microscopy of cells that have undergone extensive chemical extractions, and critics claim the resulting matrix is an artifact of these preparation techniques. Using x-ray microscopy, researchers can examine hydrated, whole cells and examine nuclear architecture without subjecting the cells to the chemical processing steps required for electron microscopy. Therefore, x-ray microscopy has the potential to be very instrumental in addressing this controversy. Ultimately, with the use of stereo images and tomographic reconstructions of cells, researchers will be able to obtain unique, three-dimensional information about protein localizations with respect to cell architecture in hydrated cells at 4 to 5 times the resolution of light microscopy. They will be able to examine the distribution of these proteins in normal cells and compare these data with the patterns of distribution of that same protein in tumor cells to elucidate structural differences that may contribute to, or be the product of, tumorigenesis.

4. DISEASE PREVENTION

New Sensor Provides Instant Litmus Test for Pathogens (LBNL). A new class of colorimetric sensor materials has been invented that makes it possible to instantaneously and inexpensively detect a wide range of biological toxins and common disease-causing organisms. Building on earlier discoveries in their laboratory, researchers at the Lawrence Berkeley National Laboratory have developed a thin film consisting of receptor molecules attached to a film of linked diacetylene molecules. The film transmits blue light. The surface receptor molecules are designed to very selectively bind specific pathogens causing the film molecules to reorganize and the film to turn red. Pathogens thus far detected with good sensitivity include an influenza virus, cholera toxin, botulinum toxin, and the *E. coli* toxin produced by the bacteria responsible for 200 deaths per year in the United States alone, as noted in the recent contamination of fruit drinks and fast food hamburgers. Existing tests for all of these pathogens require at least a 24-hour culture. With further development (150 companies have inquired) the sensors can be

incorporated into inexpensive food packaging materials such as plastic, paper, or glass and portable detection devices. Future applications of the technology are envisioned for other bacteria, viruses, illicit drugs, waste chemicals, and pesticides.

First Results of X-Ray Microscopy of the Structure of Parasitic Metazoa (LBNL-ALS). In the first stages of a project to study the structure of parasitic metazoa using the high-resolution x-ray microscope (XM-1) on beamline 6.1.2 of the Advanced Light Source, Lawrence Berkeley National Laboratory researchers compared structural information obtained by electron microscopy and confocal optical microscopy of the larvae of the parasitic nematode *Trichinella spiralis* with that of x-ray microscopy. *T. spiralis* is a parasite that infests the intestines of various mammals; it causes trichinosis in those who eat undercooked, infected pork, when larvae of the nematode travel through blood vessels and become encysted in muscle. X-ray microscopy images were also made of heartworm (*Dirofilaria immitis*), which is a filarial worm transmitted by mosquitoes and parasitic in the heart and associated blood vessels of dogs and other canids. The two samples represented multicellular organisms which are much larger in size and of more complex internal morphology than the organisms which have hitherto been examined by soft x-rays. The small size of many parasitic organisms requires the use of electron microscopy for adequate elucidation of their structures. While both transmission and scanning electron microscopy can provide complementary results that allow a considerable degree of structural correlation, each technique has its inherent limitations. Soft x-ray microscopy examination of the principal stage of the life cycle of *T. spiralis* indicated the presence of certain organ primordia in the mature (muscle larvae) much more readily and clearly than with conventional light microscopy. The soft x-ray images of these specimens also provided a three-dimensional representation of intercellular relationships and arrangement which cannot be obtained either by scanning or transmission electron microscopy. The new features revealed in the *T. spiralis* newborn larvae included a mid-body ganglion-like structure with axon-like extensions of individual cells, a structure which appeared to be a primordium of the ovary--an unprecedented degree of organ development and organization for this larval stage, and the arrangement of the posterior part of the digestive tract. In the microfilaria of the dog heartworm it was possible to observe cells of the amphidial nerves and an apparent network of interconnecting nerve cells throughout the length of the microfilaria. These preliminary results indicate that soft x-ray microscopy is an excellent tool for morphological studies, at the cellular level, of a variety of biological organisms.

Intracellular Structures of Normal and Aberrant Plasmodium Falciparum Malaria Parasites (LBNL-ALS/Monash U., Australia). In collaboration with members of the Center for X-Ray Optics, parasitologists at LBNL's Life Sciences Division have also been using the high-resolution x-ray microscope (XM-1) at the Advanced Light Source to study structural development of the malaria parasite *P. falciparum* in normal and genetically abnormal erythocyes (red blood cells) and in infected erythocycles treated with cysteine protease inhibitors, which are potential chemotherapeutic agents. *P. falciparum* is a particularly virulent organism responsible for about 45 percent of the cases of human malarial infection each year. Currently, malaria is third behind tuberculosis and measles as major killer diseases caused by a single organism with almost 1 million deaths per year, mainly women and children. There is no

effective vaccine, and drug resistance is growing rapidly. Better understanding of the process of intracellular parasite maturation and the interactions between parasites and host erythrocytes can contribute to efforts to devise novel approaches to control this deadly disease. Associations between intracellular organism and host cells are complex and particularly difficult to examine. With improved resolution and unique contrast from photoelectric absorption in x-ray microscopy, images show unusual features or structures not detected by other forms of microscopy. Image contrast is generated by differences in photoelectric absorption by the atoms in different areas (subcellular structures) throughout the sample. Absorption due to carbon predominates.

In the present study the researchers found previously undetected aberrations in the structures of the parasites that developed in the presence of cysteine inhibitors. In addition, they provided the first direct evidence for an effect of a mutation in an erythrocyte skeletal protein on morphology of intraerythrocytic stages of *P. falciparum*, an effect they showed may persist into the next generation in subsequent infection of normal red blood cells. In sum, investigations in normal red blood cells enabled the researchers to recognize anomalies in parasite structures resulting from growth under unfavorable conditions. X-ray microscopy facilitated detection of newly elaborated structures in the cytosol of fixed, unstained, intact erythrocytes, redistribution of mass (carbon) in infected erythrocytes, and aberrant parasite morphology. In infected erythrocytes treated with cysteine protease inhibitor, high concentrations of material were detected in abnormal digestive vacuoles and aggregated at the parasite plasma membrane. The researchers demonstrated that an abnormal host erythrocyte skeleton affects structural development of parasites, and that this aberrant development can be detected in the following generation when parasites from red blood cells deficient in protein 4.1 infect normal erythrocytes. This work extends the current understanding of the relationship between the host erythrocyte membrane and the intraerythrocytic malaria parasite by demonstrating for the first time that constituents of the erythrocyte membrane play a role in normal parasite structural development.

Enhanced NMR Technique for the Study of Biological Processes (LBNL). With support from the Office of Basic Energy Sciences and the Office of Biological and Environmental Research, a research team led by Alexander Pines and Thomas Budinger at the Lawrence Berkeley National Laboratory (LBNL) has employed advanced techniques in nuclear magnetic resonance (NMR) spectroscopy and magnetic resonance imaging (MRI) to time resolve gas exchange processes across human red blood cell membranes. This type of application had been limited by lack of required sensitivity. While researchers at Princeton University had developed a technique using xenon gas that increased the sensitivity one thousand fold, its general use in in vivo and in vitro studies had been limited because the xenon polarization decayed in a matter of seconds due to interactions with oxygen and other molecules. The LBNL researchers overcame this difficulty by developing a multi-step procedure to concentrate the polarized xenon at the predetermined target area before the enhanced signal decayed. The spin-polarized xenon is frozen at liquid nitrogen temperature and then dissolved into a simulated blood plasma solution. The xenon-containing solution is then injected near the biological structure of interest, thus minimizing the loss of polarization during the "delivery" process. In a model study, the team was able to measure the rate of uptake of xenon into human red blood cells. Since it has already been shown

that xenon can be safely introduced into humans, this technique has potential for studying a wide variety of physiological phenomena in the blood system and in the heart, lungs, brain, and other organs. The researchers plan to investigate how xenon behaves when carried by various lipid based structures to specific types of cells or matrix proteins associated with processes such as cancer.