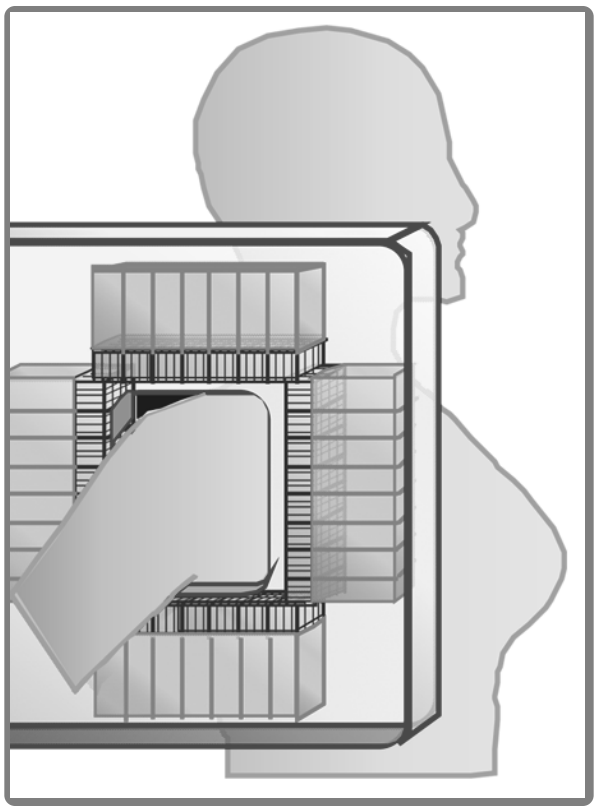
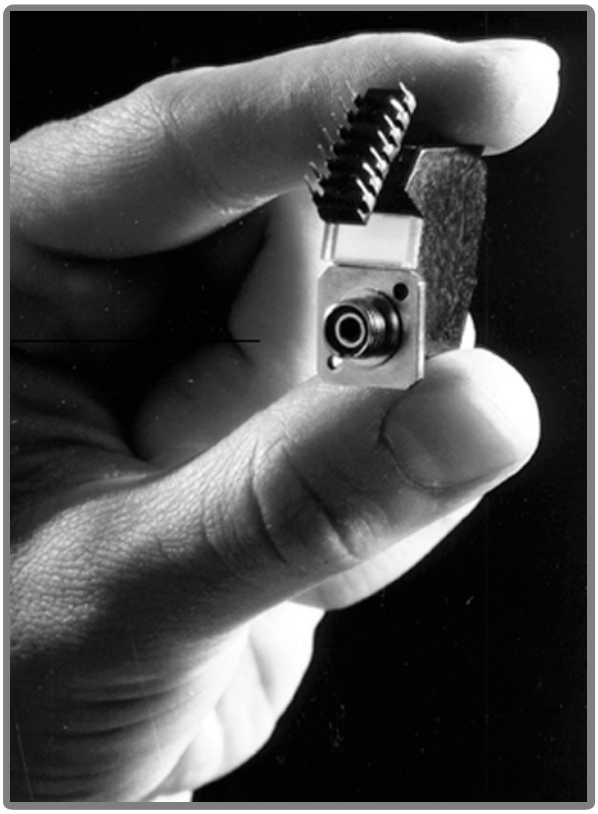
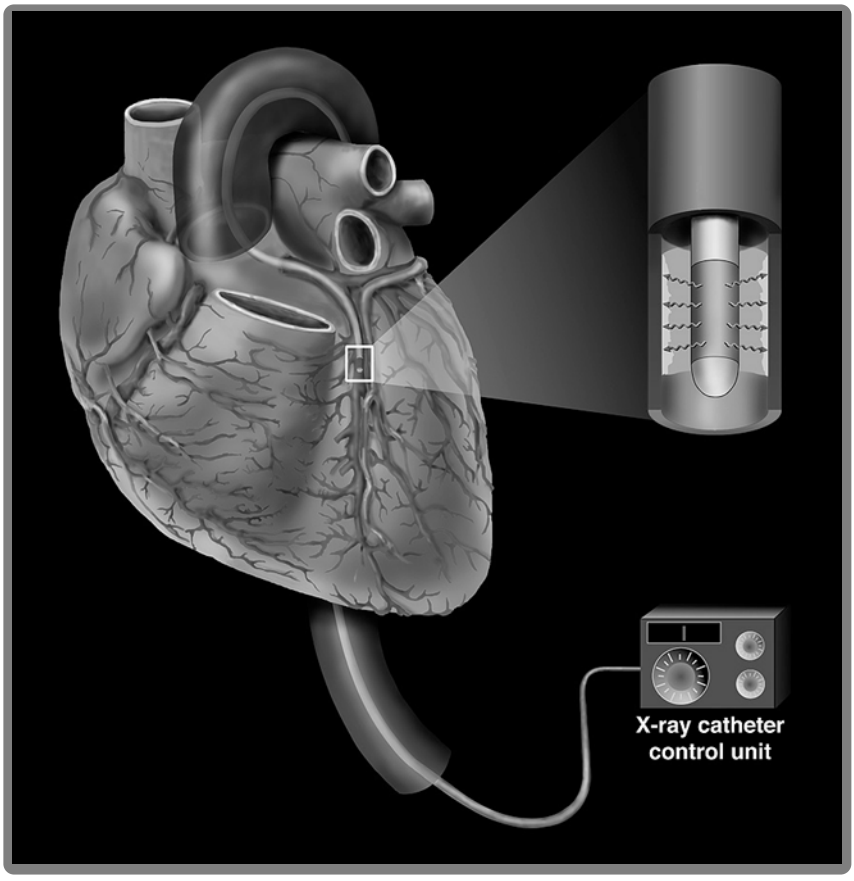


Biomedical Engineering Research

at DOE National Labs





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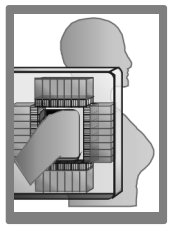
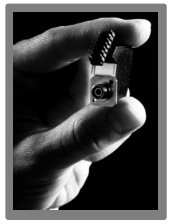


Axillary Node Positron Emission Tomography (PET)

The patient's arm is placed through the "ring" which is positioned so that the axillary nodes are near the center of the ring. The top and bottom detector arrays are movable and their spacing adjusted to be as close together as possible. The detector is divided into 30 modules, each a 2.5-cm cube. Research at the Lawrence Berkeley National Laboratory.

X-Ray Catheter

Working jointly, Interventional International Corporation (IIC) and Lawrence Livermore National Laboratory (LLNL) personnel developed a prototype miniature x-ray tube attached to the end of a shielded electrical cable. The x-ray catheter was developed for the treatment and prevention of arterial restenosis following angioplasty. The use of the catheter inhibits smooth muscle cell growth at the injury site through the use of ionizing radiation. Research at Lawrence Livermore National Laboratory.



Toolbox for Micro-Optical and Microassembly Systems

Researchers in the Engineering Technology Division of the Oak Ridge National Laboratory (ORNL) are developing several types of devices to be used in assembling miniaturized biosensors. Research at Oak Ridge National Laboratory, Center for Biotechnology.

DOE/SC--1999-1

Biomedical Engineering Research at DOE National Labs

Date Published
March 1999

Prepared for
U.S. Department of Energy
Office of Biological and Environmental Research
Office of Science
Washington, DC 20874-1290

Prepared by
U.S. Department of Energy
Office of Scientific and Technical Information
Oak Ridge, TN 37830

Contact for queries about this publication:

Office of Biological and Environmental Research
Office of Science
United States Department of Energy
Washington, DC 20874-1290

Available to DOE and DOE Contractors from the Office of Scientific and Technical Information, P.O. Box 62, Oak Ridge, TN 37831; prices available from (423) 576-8401.

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Preface

Biomedical Engineering is the application of principles of physics, chemistry, and engineering to problems of human health. It is a scientific endeavor that has been a critical part of the activities of the DOE National Laboratories since 1947.

Dr. Shields Warren, a pathologist from Harvard University, was appointed the first director of biology and medicine in the Atomic Energy Commission in October of 1947. He had the vision to develop a formal research program outside the restricted scope of industrial health and safety. It was immediately recognized that the National Laboratories were a source of medically important radioisotopes both for use as research reagents and as a potential weapon against cancer. By the end of 1947 almost 2000 deliveries of iodine-131 and phosphorus-32 were made to laboratories and hospitals. During the next decade, new radioisotopic tracers were generated under controlled conditions with cyclotrons and reactors. The new field of radiopharmacy (i.e., attaching radionuclides to biologically active molecules and studying their activity) was greatly promoted by work in the National Laboratories. The development of instrumentation to detect the cellular and total body distribution of these new radiotracers became, and continues to be, a major activity of the National Laboratories as well. The results have been spectacular—there is no major hospital in the world that does not rely heavily on technology and instrumentation that was developed in part in the DOE National Laboratories.

During the past two decades, the bioengineering programs in the National Laboratories have been the beneficiaries of the rapid advances made in nuclear physics, nuclear engineering, nuclear chemistry, and molecular biology. Research on the medical applications of synchrotron light sources, lasers, mass spectrometry, high field magnets, microfabricated machines, biosensors, DNA chips, to name a few, is ongoing in many of the Laboratories. The large number of talented scientists and extraordinary technologies have made the National Laboratories a national resource for biomedical engineering. The specialized resources at the National Laboratories enable science that cannot be done at universities or industry.

This inventory of biomedical engineering projects in the National Laboratories was compiled in January 1999 and will be updated yearly. We hope that the identification of investigators and specific areas of expertise in each Laboratory will stimulate networking and research collaboration among scientists in the DOE as well as between DOE investigators and colleagues in Universities and in industry.

In reading this compilation of research projects, I hope that the reader comprehends the extraordinary breadth and depth of the projects in the biomedical engineering enterprise in the National Laboratories. These programs are supported by different offices across the DOE. The common denominator is high-quality scientists who see the direct application of their work to advance the Nation's health.

The DOE National Laboratories prepared the information contained in this inventory. Members of the OBER Bioengineering working group—Dean Cole, Peter Kirchner, Prem Srivastava, and particularly Larry James—compiled and organized the material for this publication.

Michael V. Viola, MD
Director, Medical Sciences Division
Office of Biological and Environmental Research
Office of Science
U.S. Department of Energy
Germantown, M.D.

Contents

Biomaterials and Biomechanics

This section lists research in the area of biomaterials and devices, including the development of novel tissue, organ replacement, and device technologies that are designed to perform ideally in their respective biological environments. Also included are projects in biomechanics, including investigating the role of force, deformation, and motion of molecules, DNA, genes, genetic circuits, cells, cellular matrices, tissues, and organs.

1. Biomechanical and Clinical Data Incorporated Into Design of New Ergonomic Pointing Device

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Work-related musculoskeletal disorders (MSDs) now account for 62% of occupational illness (BLS 97). The total economic impact of MSDs was >$50 in 1995. Computer-related MSDs have been reported to increase ten-fold within the past 5 years, especially among heavy mouse users. Intensive pointing device (PD) use is observed with graphic illustration, programming and spreadsheet work at LLNL. This project will integrate LLNL's expertise in ergonomics, occupational biomechanics and computational modeling to design of a "biomechanically rational" computer PD and unique measurement tools. For this project, we have developed several biomechanical tools and techniques to accurately quantify extreme joint postures, muscle fatigue and force and repetition among graphic illustrators, CAD/CAM operators and programmers at LLNL. Biomechanical experimental results are then used to generate a pilot simulation of the interaction between the thumb and the pointing device to predict muscle tendon overuse. Data from the field, biomechanical experiments, clinical experience, and computational modeling will be integrated into the new pointing device design to address high fingertip/pinch grip force exertion during PD use and stressful joint postures during PD operation. The study is leading to a better understanding of upper extremity biomechanics during pointing device usage. Our biomechanical instrumentation can be used as an exposure assessment and training tool for ergonomic injury prevention. The new pointing device will benefit computer users and potentially reduce injury costs to US industry.

(Supported by LLNL internal funding)

2. Carbon Based Prosthetic Devices

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Athroplasty is a common treatment for arthritic disease and trauma. Approximately 300,000 to 400,000 total hip replacements (THR) are performed per year worldwide, and a similar number of knee replacements. The number of patients receiving THR's is expected to increase as the population as a whole ages. These operations have been successful in relieving preoperative pain, decreasing disability and improving the quality of life for patients. Despite the success of these operations there have been problems with the mechanical loosening of cemented implants. The problem has been associated with cement breakdown, loosening, and decreases in bone density due to stress shielding by stiff metallic components. It is widely recognized that a cementless fixation combined with a low modulus material could greatly extend the life of prosthetic devices. Carbon materials uniquely meet the required properties for a solution in terms of mechanical stiffness, direct bone apposition and biocompatibility. The biocompatibility of isotropic pyrolytic carbon is well established and has been used for over 25 years for artificial heart valves. Direct bone apposition to pyrolytic carbon, providing a cementless fixation has been demonstrated. Bone ingrowth into the surface will provide a mechanical interlock at the interface. This program is also investigating the use of diamond-like carbon and carbide coatings for wear resistance on articulating surfaces.

(Supported by CRADA with Ascension Orthopedic, Inc., a Delaware corporation, and DOE Small Business Initiative program funds.)

3. Biomaterials

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The Ceramic Processing Group at ORNL has developed the gelcasting process, a method of fabricating ceramic bodies by adding organic monomers to a suspension of ceramic powder and polymerizing it to form a green body of ceramic filled polymer-solvent gel. The Ceramic Processing Group is involved in a CRADA with a prominent biomaterials company to develop and commercialize net shape forming methods for directly creating dense bioceramic implants. Bioceramics, in both dense and porous forms, are increasingly being used in bone replacement surgery because of their well established biocompatibility. Certain implants, because of their small size and/or complex shape, are costly to manufacture by the traditional approach of machining from dense ceramic billets. Our gelcasting process allows us to cast implants to net shape, thereby increasing yield while decreasing production costs. Additionally, green gelcast parts (those that have not been sintered to full density) are strong enough to withstand the stresses of machining. Green machining can be accomplished at significantly increased speeds with less expensive tooling and with increased yields, allowing for prototypes or custom implants.

(Supported by CRADA)

4. Disposable Micromechanical Sensors for Biomedical Applications

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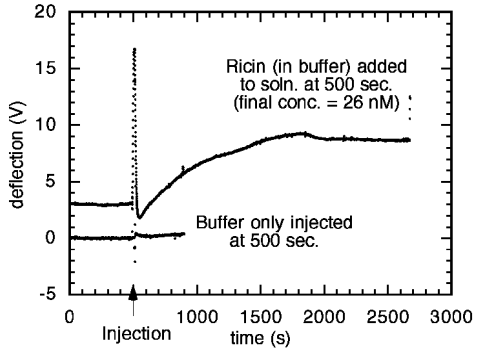
ORNL researchers have patented and developed a spectrum of chemical and biological sensors based upon the microcantilever springboard platform. Microcantilevers, such as those used by atomic force microscopes, have been demonstrated as a universal platform for real-time, *in-situ* measurement of chemical and biological analytes. Based upon micro-electro-mechanical fabrication of silicon combined with the sensitivity utilized in atomic force microscopy, the technique promises to revolutionize applications where multiple analytes need to be economically monitored. Advantages of these extremely sensitive devices include miniature size, simplicity, low power consumption, potentially very low cost to manufacture, inherent compatibility with array designs, and the ability to operate in biologically relevant fluids. Micromachining technologies currently available could be used to make multielement or multitarget sensor arrays involving hundreds of cantilevers without significantly increasing the size or complexity, or cost of an overall sensor package. A robust multielement, disposable sensor array can be constructed for sensitive detection of antigens, proteins, enzymes, DNA sequences, blood gases, and specific ions such as Na, K, and Ca in biological fluids. By monitoring the resonance response of the cantilever, analytes can be detected with unprecedented accuracy. Figure below shows detection of ricin sensitivity in the parts-per-trillion range using an antibody-coated microcantilever. Other demonstrated application include detection of DNA hybridization, bacteria, and glucose. With this technology, economical multicomponent sensing can be accomplished in real time using a single, disposable micromachined chips.



(Supported by OBER, CRADA)

5. Bioactive Coatings

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Pacific Northwest National Laboratory developed the surface-induced mineralization process (SIM), a low-temperature, aqueous process that can coat many different substrate materials and surfaces. Bioactive calcium phosphate coating has been grown on smooth and porous titanium metal and titanium alloys. The coating is the mineral phase of bone, which provides a compatible interface between the bone and the implant. Preliminary tests show the SIM coating was stronger than a plasma-sprayed coating and histological evaluations indicated that the rate of new bone formation was superior to the plasma-sprayed counterpart. The calcium phosphate coatings have been and are undergoing preliminary in vitro and in vivo [animal] tests. Proteins, such as growth factors, incorporated into the coating maintain their viability because of the low temperature. More information is available at http://www.pnl.gov/medical/thera/biocoat.htm

(Supported by DOE CRADA with 3I)

6. Protein-Crystal Molecular Recognition in Biomineralization

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Acidic proteins found in mineralized tissue act as biology's crystal engineers. Their activities are responsible for the material properties of hard tissues, and they directly control the hierarchical architecture of these tissues. However, despite their importance in such fundamental physiological processes as bone and tooth formation, there is remarkably little known of the protein structure–function relationships which govern crystal recognition. The primary goal of this program is to obtain a molecular description of the structure–function relationships used by small acidic proteins in the crystal engineering of hydroxyapatite and calcium oxalate (the principle mineral phases of bone/teeth and kidney stones, respectively). The disruption of normal biomineralization processes can lead to pathological mineralization or demineralization, such as in artherosclerotic plaque formation, artificial heart valve calcification, kidney stone build-up, dental calculus formation, or bone and tooth demineralization. A better understanding of the biomolecular mechanisms used to promote or retard crystal growth could provide important design principles for development of calcification inhibitors and promoters in orthopedics, cardiology and dentistry.

(Supported by National Institutes of Health through the University of Washington)

7. Interfacial Interactions of Biological Polymers with Model Surfaces

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The mechanisms that control the adsorption of biological polymers onto surfaces are not well understood. The objective of this project is to further the understanding of interactions of biopolymers with surfaces. By understanding these interactions, it should be possible to design systems that exploit and control biopolymer adsorption processes and prevent biofouling of medical devices. This project is applying state-of-the-art methods such as molecular beam epitaxy, chemical vapor deposition and self-assembling monolayers to construct surfaces with controlled properties. We are also developing CVD methods to produce controlled surfaces of the biologically relevant calcium oxalate, carbonate, and phosphate systems. Biological polymers of human serum albumin, Protein G and fibrinogen are used in the adsorption experiments. State-of-the-art techniques (neutron scattering and reflectometry, quartz crystal microbalance, liquid chromatography/mass spectroscopy and atomic force microscopy) are used for in situ study of adsorption kinetics, isotherms and protein conformation. Solid-state NMR experiments will identify the specific protein residues that are interacting with the surface.

(Supported by DOE CRADA with Ross Products, A Division of Abbott Labs)

8. Novel Processing Methods for Producing Bone Augmentation and Replacement Materials

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Ceramic materials are used to restore bone to form and function by serving as a substitute for natural tissue that is lost due to disease, accident, or surgery. Recent interest in this area has focused on the use of composite materials of either tricalcium phosphate (TCP) or hydroxyapatite (HAP) combined with thermoplastics, collagen and other polymeric materials used as bone scaffolds, fillers, and reconstructive agents. The purpose of this project is to investigate the feasibility of combining these types of materials and a unique method for producing ceramic components called near-net shape forming to fabricate both quick setting and dense preformed implantable tissue substitutes.

(Supported by Army Medical Research and Materiel Command)

9. Interaction of Proteins with Surfaces

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This project is focused on developing experimental methods utilizing selective proteolysis to compare structural features of proteins subjected to surface-mediated unfolding with their native conformation in solution. Existing computer models of protein folding will be extended to include interactions with surfaces and will be validated by comparison with experimental data. Through this combination of theory and experiment, we will advance the fundamental understanding of how proteins interact with surfaces and the interplay between protein environment and conformation. This insight, particularly as it relates to large modular proteins involved in cell adhesion and blood clotting, will enable the design of materials for biomedical applications to control protein-surface adhesion due to unfolding.

(Supported by PNNL LDRD funds)

10. Biocompatibility of Surfaces

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The objective is to advance the fundamental understanding of how interfaces control biological responses, i.e., how interfacial properties affect protein adsorption and conformation and how protein adsorption, in turn, controls cell adhesion and growth. These studies will lead to strategies for tailoring polymeric materials and implant surfaces to promote specific biological responses. We have focused on protein and cell growth systems to learn how to form anti-thrombogenic surfaces. In the first year we examined endothelial cell growth and competitive protein adsorption as a function of surface properties. Next we examined competitive cell growth between endothelial cells and platelets. Since protein adsorption and conformation are key to cell adhesion and growth, chromatographic studies are used to begin to address the kinetics of protein adsorption and coupled mass-spectral studies to understand time dependent changes in conformation.

(Supported by PNNL LDRD funds)

11. Architectural Design of Three-Dimensional Scaffolds for Biological Applications

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This project developed a range of forming processes that provide a platform for the construction of three-dimensional organic and inorganic scaffolds. These scaffolds will have wide use as cell supports, bone fillers, and tissue regeneration templates. Three different approaches will be taken: 1) tape casting and

calendaring, 2) bi-continuous reverse microemulsions, and 3) rapid prototyping. Each technique offers a unique route to forming structures with controlled pore size and structure. These techniques are versatile in that many different materials can be formed. This program will address the fundamental aspects of forming structures with controlled three-dimensional network and porosity. The effects of binder systems, solids loading, and the characteristics of the water–oil–surfactant system on strength, porosity, and resorbability will be evaluated.

(Supported by PNNL LDRD funds)

12. Engineered Biomaterials for Accelerated Wound Care

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A systematic approach is being used to evaluate therapeutic peptides and peptide-bearing scaffolds ability to promote cell migration and proliferation across dermal wound beds. Peptide-scaffold devices will be developed and tested in an in vitro test system to quantify keratinocyte (epidermal cell) and fibroblast (mesenchymal cell) migration across a simulated wound bed. Efforts in FY98 will focus on construction of the in vitro test system to quantify cell migration. In addition, a skin cell (keratinocyte and fibroblast) cultivation capability will be developed at PNNL. Future efforts will be directed towards the development and testing of therapeutic peptide-scaffold devices to promote skin wound healing. Promising devices will be further tested in animal systems in FY99 and FY00.

(Supported by PNNL LDRD funds)

13. Reversible Gels for Chondrocytes Cell Culture and In Vivo Cartilage Regeneration

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The application of reversible gels for chondrocyte (cartilage forming cells) cell culture and development of injectable formulations for cartilage regeneration will be investigated. The rationale of using reversible gels for chondrocytes cell culture is related to the fact that reversible gels offer a possibility of a three-dimensional culture environment that, in case of chondrocytes, is essential for the preservation of their in vivo phenotype. Also, the reversibility of the gelation process allows for easy recovery and harvesting of cultured cells by simple dissolution of the polymer gel by lowering of the temperature. The second part of the project involves the development of injectable formulations for autologous cell therapy of cartilage defect regeneration and is based on the fact that reversible gels may exhibit a phase transition exactly at body temperature. Upon injection of the chondrocyte suspension in a geling polymer solution into a defect, a soft extracellular matrix suitable for chondrocyte proliferation and growth is created.

(Supported by PNNL LDRD funds)

14. Dissolvable Stents

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Vascular reconstruction is an important means of life-saving treatment in trauma injuries and in vascular disease. The reconnection of arteries and veins has a high failure rate due to stenosis at vascular anastomoses, thrombosis at the reconnection site, localized infection, and the mechanical stress of vessel wall contractions. Additionally, the repair of small arteries is tedious and technically difficult. Considerable work has been focused on development of

materials to replace missing tissue, but little work has been conducted on the development of materials that will enhance anastomosis or serve as a template for vascular healing. The aim of this project is to develop and test material(s) that serve as templates for tissue reconstruction and meet the following four criteria: (1) enhance healing, retard thrombosis and stenosis; (2) are bioresorbable to prevent continued stimulation of cellular hyperplasia or localized toxemia; (3) provide a porous surface either initially or by dissolution for enhanced tissue attachment; and (4) be mechanically robust so as to be fabricated into a thin-walled tube that will tightly fit into the lumen, maintain normal blood flow, and withstand the normal peristaltic action of the vessel. The ultimate goal will be to develop a resorbable device that can be delivered via minimally invasive surgical techniques.

(Supported by National Medical Technology Test Bed)

15. Development of Advanced Lower Limb Prosthetics

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This project advances the development of the Rolling Joint Prosthetic Foot and Ankle, a prosthesis that more nearly simulates human gait than other prostheses. The Russian weapons laboratory VNIITF is involved in the design of the controlling mechanical bands which provide the controlled, but variable, force exerted on the ankle. Stress analysis, improved band materials and durability testing are provided by the Russians to further the development which is expected to lead to a product for the partnering US company. The project fulfills the special niche of simultaneously meeting the non-proliferation mission of DOE by diverting Russian weapons researchers to activities of civilian need and the health research need of NIH by advanced development of this prosthesis. Utilizing stress analysis, the band attachment mechanism has been redesigned and extensive durability testing results have been favorable. A new product is anticipated within about two years. The project (FY97) is ongoing

[Supported by DOE NN and NIH NICHD/National Center for Medical Rehabilitation Research (NCMRR)]

16. Topical Hyperbaric Oxygen Treatment Pressure Indicator

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Numotech is in the process of introducing its proprietary and highly effective Topical Hyperbaric Oxygen Treatment (THOT) process for healing wounds and sores. Skin and ulcer wound care product sales totaled approximately $1.4 billion in the US in 1993, and are projected to grow to $2.5 billion in 1998. Over one million Americans are afflicted annually with pressure sores costing approximately $6.5 billion for treatment. Clearly, there is a demand for an improved, more user-friendly, and more cost-effective skin ulcer and wound care treatment system. The improvements proposed in this CRADA will further enhance the delivery and monitoring of the THOT regimen and will result in faster wound healing times, fewer patient complications, lower treatment costs, and an enhanced commercial introduction of the process. The pressure indicator should be distributed to hospitals within 1999, and the automated system should be distributed to hosptials in 2001. Distribution to home care setting should be by 2002.

[Supported by Numotech Corp. (CRADA)]

17. Computational Engineering of Sensor Materials

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This work developed the “shuffle-and-screen” algorithm to quickly screen a database of small molecules on a computer and predict which ones will bind to the target. It is being applied to the development of novel sensor materials. A necessary step in sensor design is to discover a material that binds and molecularly “recognizes” a target biomolecule. Combinatorial Peptides (CPs) are a new class of

molecular recognition materials (MRM), based on small molecules that can be engineered to bind tightly and specifically to a target analyte. They have a significant advantage over antibodies and enzymes in that they are much more rugged, cheaper, and more versatile. However, identifying highly selective CPs can be time-consuming, since potentially 10^{12} CPs may be possible, but only 10^5 compounds can be tested at a time. We use computational modeling of molecular recognition and have developed a unique software algorithm, "shuffle-and-screen", that will greatly enhance the efficiency of the sorting process to discover highly select CPs, by predicting on the computer the appropriate subset to test. Our algorithm takes advantage of inherent redundancies in the combinatorial compounds, reducing the search time to examine all possibilities from 37 years to less than a day. The shuffle-and-screen algorithm was designed to be useful for drug discovery as well as the design of sensor materials. Many drug companies are now using combinatorial libraries to search for new lead compounds and improve existing ones. As with peptides, there are often many more compounds possible in these libraries than can be practically synthesized. The shuffle-and-screen algorithm can be used to speed this process, by examining a "virtual" combinatorial library of all possible compounds on the computer, and predicting which subset is the most promising to synthesize. This project (FY98) is ongoing.

(Supported by Laboratory Directed Research and Development)

18. Chemical Recognition of Pathogens

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Development of artificial bacteria and viruses for a replacement to the biological ASTM 1671 test. This test addresses the viral penetration of pathogens through healthcare related garments and wraps. Spectral signatures are customized for detection using multispectral fluorescence. This product is currently in use in K-C mills and the resulting fabric (surgeons gowns) went on sale 10/1/98. This project develops a candidate point and standoff detector of biologicals and chemicals. The resulting garments improve the safety and comfort of healthcare and biological workers. Time Frame: FY96–FY99

[Supported by Kimberly-Clark Corporation (CRADA)]

19. Portable Neutron Spectrometer

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The goal of this project was to demonstrate the feasibility of a multilayer neutron spectrometer consisting of concentric layers of boron-loaded organic scintillator. This spectrometer would have the functionality of the Bonner sphere spectrometer and, at the same time, retain the portability and ease of use of a rem-meter. This instrument would combine the best features of the above devices while eliminating their shortcomings. The key features enabling this performance are as follows: (1) each layer acts both as a separate thermal neutron detector and lightguide, (2) all layers combined form a spherical neutron moderator, (3) the set of layer energy responses is suitable for neutron spectra unfolding. The project could be continued by building and testing additional components of the system.

(Supported by Stanford)

20. Microdrop Fluid Ejector

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For an exotic particle search at SLAC, we have had to design and construct fluid drop generators able to reliably produce drops orders of magnitude smaller in volume than those produced either in current inkjet printers or in commercial laboratory drop ejectors used for microdispensing reagents. A number of biotechnology companies have shown interest in the SLAC drop ejector because its capability to

significantly reduce the volume of controllably dispensed fluids can increase the detection sensitivity and reduce the costs of their products. These companies develop techniques in disease diagnostics and drug discovery working with gene chip microarrays, protein-detection microdots, precision dispensing of hyperprecious samples, submicroliter combining of reagents, and combinatorial chemistry. We are currently working on continuing refinement and control problems of our microdrop technology and seeking internal funding for a part of that work. Medical categories are prevention, diagnosis, monitoring, treatment, and public health.

(Supported by DOE)

21. Characterization of the Low Energy Photon Response (6-30 keV) of Different Thermoluminescent Dosimeters

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There is a need for low-energy x-ray dosimetry in radiation protection and medical diagnostics (e.g., mammography) and therapy (e.g., treatment of skin disorders with 5-20 keV x-rays). Most commercially available dosimeters are either not designed to respond well to photons with energies below 20-30 keV, or do not have a well-characterized low-energy x-ray response. We have characterized the response of various passive dosimeters using monoenergetic synchrotron radiation. In the treatment of superficial lesions such as skin cancer, with radiation, it is important to know the variation of dose with depth in tissue. With this knowledge, the appropriate radiation energy can be chosen to deliver the needed dose to the entire lesion and spare as much as possible the underlying tissue. These measurements in monoenergetic beams can be chosen to validate calculations that integrate the dose distribution over an energy spectrum for clinical applications. Knowledge of backscatter factors is essential in both diagnostic radiology and radiotherapy for the determination of the absorbed dose (surface) to the patient. The results will contribute to the understanding and evaluation of parameters that account for the change in response of an ionization chamber between in-air and in-phantom measurements in use in radiotherapy dosimetry protocols.

(Supported by DOE)

Biological, Chemical, and Genetic Sensors

This section lists projects describing research and development of chemical sensors, biosensors and biochips by converging several of the analytical, chemical, physical, electrical, biological, computational, nano/micro/fabrication, and bioengineering technologies on chip substrates, wave guides or optical fibers. Included in the portfolio are devices which have technological advancements incorporated to detect and analyze, with high sensitivity, the changes in temperature, acoustic vibrations, vapors, optical signals, spectral frequencies, rheology, atomic force, and molecular structures. The technologies are described to have many applications including study of chemical, biological and genetic matters for detection of pollutants, pathogens, infections, gene mutations, and human exposures and diseases.

22. Genetic Reader

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DNA sequencers could play a pivotal role in efforts to sequence the genetic makeup of humans, called the human genome. Ultimately, the technology could help treat such diseases as AIDS and cancer, or lead to important advances in the fight against Alzheimer's disease, muscular dystrophy, Down syndrome and other genetic disorders. The key to Yeung's device is that it can read DNA sequences much quicker than other systems. For example, it would take conventional equipment up to 1,000 years to sequence the entire three billion base pairs that make up the human genome. When scaled up, Yeung's device could produce raw data fast enough to read the entire genome in 68 days. This technology has been recognized with a R&D 100 Award in 1997.

(Supported by OBER)

23. Biochips for Gene Research, Medical Diagnostics, Pharmaceuticals, Disease Treatment, Microbial Identification, Environmental Restoration, Agricultural Products, and Crime Prevention

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In June 1998, scientists at Argonne and the Engelhardt Institute of Molecular Biology in Russia announced a 5-year, $18-million research effort with Motorola Incorporated and the Packard BioScience Company (Packard Instrument Company) that will speed the pace of advances in gene research, medical diagnostics, pharmaceuticals, disease treatment, microbial identification, environmental restoration, agriculture, and even crime detection. This joint-research agreement is one of the largest biotechnology agreements ever signed by a DOE laboratory. Plans to develop and market a biological microchip, or biochip, and related technologies will permit faster and more efficient detection of mutations in genetic information. This information is encoded in DNA, the building block in human genes that makes each of us unique. The use of biochips will accelerate the decoding of genes in the human genome, the complete set of chromosomes in a person, nature's set of blueprints and operating instructions which consists of as many as 100,000 genes. Using robots to manufacture chips that unravel DNA sequences provides an automated approach. This approach facilitates the decoding of

"phrases" of DNA, rather than reading the code one "letter" at a time. By combining biochips with robots and computers, biologists can detect one genetic variation among three billion DNA bases in minutes. Thousands of biochemical tests will be able to be performed routinely in less than an hour. Conventional methods take days. Biochips can identify which genes in a cell are active at any given time and how they respond to changes. This capability allows the physician to determine what genes might be the cause of a particular disease, and provides the pharmacologist with a basis for the design of drugs that might be useful in treatment. The result—more rational and closer coupling of disease diagnosis and treatment. The clinical diagnostics market alone will explode by billions of dollars as doctors begin to utilize these biochips to test for a wide variety of life-threatening conditions, genetic defects, viral and microbial diseases, at tremendous cost savings to consumers. Packard estimates that miniaturization and mass production will reduce drug screening costs from $5 per sample to 40 cents, or even 4 cents per sample. Initially the market will be pharmaceutical companies, research facilities, biotech firms, and academic research institutions worldwide. Ultimately, biochips will be used in hospitals and clinical laboratories. Commercialization of the biochip will impact: (1) Drug discovery and medical treatment—by identifying target genes and testing new drugs and treatments far more rapidly for possible genetic impact as in the assessment of new treatments for AIDS and tuberculosis; (2) Medical diagnostics—in the rapid identification of mutated genes that could lead to cancer, multiple sclerosis, or Alzheimer's; (3) Environmental restoration—by using biochips as highly sensitive detectors of microbial or organic pollution, thus enabling the identification of natural enzymes to be used to detoxify chemicals and digest pollutants, and of friendly genes to be used to clean up contaminated soil and water; (4) Agricultural products—by quickly and accurately detecting agricultural disease and mutation and speeding the testing of safe agricultural products; and (5) Crime detection—by making possible far more accurate and sensitive tests for crime analysis. In addition to the precommercial collaborative effort, the Argonne/Russian scientists continue to develop and refine DNA chip technologies for various applications—validation of DNA sequences, detection of genetic diseases, and identification of pathogenic bacteria and viruses. Biochips are essentially biochemistry laboratories in miniature. By attaching antibodies instead of nucleic acids to the biochips, hundreds of immunoassays on a single sample might be performed. Inserting appropriate enzymes and/or the products of enzyme activity onto the biochip would allow their use in environmental chemistry and medical forensics.

[Supported by DOE, Office of Biological and Environmental Research, Department of Defense Advanced Research Projects Agency, Motorola Incorporated, and Packard BioScience Company (Packard Instrument Company) (CRADA)]

24. Immunoassays for Actinides

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Argonne uses biological molecules to provide a way to test inexpensively for actinides in environmental and, possibly, clinical samples. Because these assays will lower the limits of background radiation over conventional radiological tests, applications range from enabling more accurate levels of determination for actinides in drinking water, to monitoring environmental contamination, and to verifying treaty constraints. DOE recently filed a patent application on this technology.

(Supported by DOE, Office of Nonproliferation and National Security, NN)

25. Gene Expression and Protein Function

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Argonne is using modified and novel gene expression technology to identify genes induced or repressed by environmental stresses, clone these genes using high-density membranes, and establish a functional role for the gene modulatory response. The Laboratory is also using genomic approaches to identify novel stress response elements. Invention reports have been submitted. This Argonne research provides a new technology and methodology for identifying functional components of the DNA sequence that regulate gene expression and genes that are differentially modulated in response to a variety of stresses.

(Supported by DOE, Office of Biological and Environmental Research)

26. Sensitive Detection and Rapid Identification of Biological Agents by Single Molecule Detection

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The techniques of fluorescence immunoassays, capillary electrophoresis (CE), and single-molecule detection (SMD) will be integrated into a man-portable system for the rapid and reliable identification of biological agents. The selective nature of antibody binding provides a highly accurate means of identification of target biological agents. The highly selective bindings of antibodies will be exploited to attach fluorescent tags. Capillary electrophoresis will then be used to rapidly separate fluorescent-labeled antibody-antigen complexes from the unbound antibodies and other components of the complex mixtures. This separation is critical in reducing or even eliminating false-positive identifications in the detection of the biological agents. The capillary will be coupled into a micro-Flow Cytometer. Laser techniques capable of single molecule detection will be used to detect the fluorescent tags. If it is successful, the proposed analytical system has uses in the areas of BW-related agent detection, environmental monitoring and heath-related fields.

(Supported by LDRD)

27. Blood Chemistry Sensors

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Researchers at the Lawrence Livermore National Laboratory are developing blood chemistry sensors that can continuously measure pH, oxygen, glucose and other analytes, such as enzyme precursors of heart attacks, and analytes which indicate stroke. Our sensors are based on a class of novel molecules that change their fluorescence properties depending on the concentration of the target analyte in the blood. These changes can be measured *ex vivo* through fiber optic links. Our near term focus is on continuous blood glucose monitoring for type I diabetes treatment. When linked to an insulin pump, these sensors will provide a bio-mechanical artificial pancreas.

(Supported by LLNL internal funding/Industry)

28. Development of a Handheld MiniPCR Instrument

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Commercial real-time PCR instruments are not suited for point-of-care or remote testing for infectious diseases in clinical and environmental samples due to

a number of limitations, particularly with respect to size, weight, power usage, speed, and ruggedness. We are currently developing, building, and testing, microchip-based spectrofluorometric thermal cyclers that overcome these limitations. One of these instruments, the Advanced Nucleic Acid Analyzer, is a portable, low power device that offers real-time PCR analysis, can be battery-operated, contains no moving optical components, and utilizes software tailored for non-technical users. Ten reaction modules and a laptop computer are housed in a protective casing, with each reaction module harboring a silicon reaction chamber with highly efficient thin-film heaters and a dedicated low energy optical system. PCR detection of bacteria has been demonstrated in as little as seven minutes. The next generation instrument will be a handheld battery-powered unit capable of performing multiplex assays. Sample preparation modules utilizing microfluidics are also being developed to replace manual pipetting.

(Supported by DOE-NN)

29. Functional Gene Expression Microarrays

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Researchers at the Lawrence Livermore National Laboratory are developing sensitive methods for measuring genetic and environmentally induced alterations in gene expression in mammalian cells. This technology is to be applied to (a) understand the roles of repair genes in disease susceptibility, (b) identify the genes critical for human meiosis and fertility, and (c) identify the early determinant events in tissues exposed to low-dose physical and chemical stresses. Preliminary results show that expression microarray technology is a promising approach for understanding the roles of large numbers of genes during normal development and in the onset of disease.

(Supported by DOE-OBER / NIH / NIEHS / University of California)

30. DNA Chip Analysis

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Recent advances in photolithography and oligonucleotide chemistry have led to the development of a high-density array "DNA chip." With this technology it is possible to simultaneously perform 65,000 individual DNA hybridization reactions. Our laboratory is collaborating with Affymetrix Corporation, a leader in this field, to develop methods for the use of this technology in bacterial identification that are both rapid and precise. We have designed DNA chips for high-throughput, sequence specific bacterial identification. Amplified PCR products indicating the presence of a particular organism are placed on the chip for hybridization. Confirmation of sequence specific to individual amplicons is determined by quantifying the intensity of hybridization to an ordered set of 20-mer probes. Integrated software compares the sequence of the hybridized product to the predicted sequence of the species-specific amplicon as a basis for a positive call. Multiple organisms may be analyzed simultaneously with an extremely high level of confidence of making positive identifications. We are also using DNA chip technology to rapidly identify any bacteria or higher order organism by detection of specific small sub-unit ribosomal sequence. Comparison with known ribosomal sequences is used to classify the unknown organisms to within a group of related species. This technology has application to human, veterinary, agricultural, and environmental diagnostics.

(Supported by FBI, other federal agencies)

31. Rapid Identification of Microbial Species

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We have developed AFLP (amplified fragment length polymorphisms) to rapidly identify microbial species to the strain (sub-type) level. The method can be used directly by comparison of newly generated profiles to an archive of previously generated archives. A comparison of similar and different fragments can also genetically place a previously uncharacterized microbe relative to those previously studied. This allows rapid identification of new pathogens by determining their relationship to other microbes. The method can be very rapid with several hours' turn-around time very realistic. We are in the process of establishing an archive of AFLP profiles for many different microbial species and strains. This program also includes development of computer software to rapidly compare new profiles to archived profiles and to automate the phylogenetic analysis. We are also using data generated from the AFLP profiles to identify DNA sequences that are unique to different strains of particular microbial species. These strain-specific sequences are used to generate PCR based methods of rapidly screening complex samples for these strains. The same method is being used to identify DNA fragments that are shared among small or large groups of species. The information from these fragments will also be used for sample characterization. However, they will provide a means of detecting and identifying a previously uncharacterized or genetically engineered microbe. Together, the methods should provide an excellent means of rapidly screening samples and identifying agents in the samples to the strain level. Such information is very important for attribution of a disease to a particular source. We are patenting the PCR primers developed from AFP profiles for *B. anthracis.*

(Supported by DOE, Security Agencies)

32. Noninvasive Intracranial Pressure Measurement System

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The noninvasive intracranial pressure (ICP) system will employ a Swept Frequency Acoustic Interferometry (SFAI) technique that has been developed and patented by Dipen Sinha at Los Alamos National Laboratory (LANL) for noninvasive identification of chemical warfare agents inside sealed munitions. The SAFI technique is extremely sensitive in determining small changes in physical and elastic properties of any material or of the contents of fluid-filled containers. In the present application, a small piezoelectric transducer is lightly applied to the patient's head and the electrical signal is swept over a range of frequencies (with wavelengths on the order of skull thickness) to excite low amplitude vibrations or wave motion within the *in vivo* skull and brain media. A second identical transducer placed nearby monitors the cranial response. The output transducer detects the resonant frequencies that correspond to resonant standing waves in the skull or in the brain (depending on frequency range). Minute changes in sound speed of the skull or brain matter or in cranial dimensions due to ICP change are monitored through the variation in resonance peak spacing in the observed SFAI spectrum. A detailed finite element model of the human head (based on the Visible Human Dataset provided by the National Library of Medicine) will be used to guide development of the prototype systems and to provide interpretation of the observed SFAI data. This modeling effort will use the LANL head model developed by William O. Wray and Toru Aida with internal LANL funds and contract support from the General Motors Corporation. The head model is based on the Visible Human dataset provided by the National Library of Medicine. It is expected that the noninvasive ICP measurement system will have applications in the field of combat casualty care as well as in non-invasive medical diagnostics in the primary care physician's office.

(Supported by U.S. Army Medical Research and Materiel Command)

33. Regenerable Enzyme Electrodes

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Thousands of different redox proteins are available from a large variety of biological organisms. Most act upon a single, specific substrate to produce a unique, specific product with the consumption or liberation of electrons. In the human body the current flow due to all redox reactions is equivalent to 75 A or more. Redox enzymes from different classes will usually donate and accept electrons from similar organic or inorganic electron mediators. Flavoproteins, for example, will usually couple to various ferrocenes, phenazines, thiazines, or oxazines. Electron mediation by quinones or pterins is common for other proteins. Still another class of non-heme iron proteins couple to viologens. If these artificial electron donors/acceptors are tethered to an electrode surface with a linker of appropriate length to reach the active site of the enzyme, electron mediation between the substrate-enzyme-mediator-electode becomes possible. By affinity for the electron mediator the enzymes orient at the surface and are adsorbed by electrostatic interactions. The presence of a specific substrate for the enzyme causes a current flow in the electrode which can be amplified, therby creating a biosensor for that specific substrate. Different enzymes of the same class become specific biosensors for their own substrate when adsorbed onto the same electrode. Spent enzymes can be easily removed from the surface by electrochemically reducing the functional groups to an anionic state and replacing with fresh enzymes when the electrode is re-oxidized. Most of the cost of such devices is in the electrode and its derivatization which, in these cases, can be repetitively re-used. Similar constructs of enzyme electrodes could be used to quantify drug titers, poisons, anesthetics, or secondary metabolites in body fluids. Measurements can be made in real time. The devices could be fabricated on a larger scale to create biofilters to remove excess uric acid or carbon monoxide, for example, from blood, or made smaller for use as implantable devices to detect and control blood sugar levels. Basic Science researchers have been issued or are affiliated with several patents in these areas. In addition to having medical diagnosis and treatment applications, the technology is of use in potential public health matters concerning food spoilage and environmental sensing and bioremediation.

(Supported by Spin-off from EERE Hydrogen Program)

34. The Molecular Analysis of Genomes by AFM

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We have precisely located sequence specific proteins bound to individual DNA molecules by direct AFM imaging. Development of this technology offers a unique opportunity to identify point mutations, verify gene promoter regions, and investigate protein modulated gene expression on intact genomic clones. Using a mutant *Eco*RI endonuclease that site-specifically binds but doesn't cleave DNA, bound enzyme has been imaged and precisely located, with an accuracy of 1% on well characterized plasmids and bacteriophage lambda DNA (48 kb). Cosmid clones have been mapped. This direct imaging approach could be rapidly developed to locate other sequence-specific proteins on genomic clones. Point mutations and small lesions could be identified and located on genomic DNA by imaging proteins, such as MutS, involved in identifying and repairing damaged or mutated regions. Transcription factor proteins that identify gene-start regions and other regulatory proteins that modulate the expression of genes by binding to specific control sequences on DNA molecules could be precisely located on intact cloned DNAs. Conventional gel-based techniques for identifying site-specific protein binding sites on DNA molecules rely on either fragment analysis, for identifying restriction enzyme sites, or, for other proteins, on gel-shift methods that only address small DNA fragments and are woefully inadequate. Conversely, AFM imaging is a general approach that is applicable to the analysis of all site-specific DNA protein interactions on large insert clones. This technique could be developed for high-throughput analysis, can be accomplished by technicians, uses readily available relatively inexpensive instrumentation, and should be a technology fully transferable to most laboratories.

(Supported by LDRD)

35. Flowthrough Genosensor Chips

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A flowthrough genosensor chip is under development at ORNL. The core of this technology is a microchannel hybridization array, containing numerous specific DNA sequences, immobilized within individual cells of densely packed straight, smooth channels traversing a thin silicon or glass substrate. When a nucleic acid sample is labeled and passed through the microchannel genosensor chip, hybridization occurs at porous cells bearing immobilized DNA probes complementary to the target sequence. The quantitative binding pattern reflects the relative abundance of specific target sequences within the nucleic acid analyte. The flowthrough chip configuration has several important advantages over flat surface DNA chips being developed elsewhere: faster hybridization kinetics, superior binding capacity, improved ability to analyze dilute solutions of nucleic acids, including both strands of a heat-denatured PCR fragment. Related technology for taking advantage of the benefits of the flowthrough genosensor includes the development of micromachining techniques for the construction of flowthrough silicon chips to complement those constructed using channel glass. A customized robotic spotting system has been developed that includes a high resolution positioning system, sapphire dispensing tips for touch-off dispensing, and, more recently, solenoid-controlled ink jets for remote droplet delivery. A prototype fluidics system has been developed that involves syringe pump-driven fluid flow, a custom chip holder attached to the stage of a Zeiss Axiovert fluorescence microscope and a CCD camera for real-time quantitative detection of hybridized fluorescent-labeled strands. A software package for intelligent selection of oligonucleotide probes for a given chip application has been developed. The flowthrough genosensor system is now being used to develop applications in the areas of genotyping and mRNA profiling, in collaboration with various laboratories. Gene expression profiling of mammalian systems, including mouse and sheep, is being pursued as well as bacterial systems for evaluating soil microorganisms as an indicator of genotoxic response in the environment. Another application being developed is high throughput genotyping. In this work miniature flowthrough genosensors are used to simultaneously analyze numerous single nucleotide and short insertion-deletion polymorphisms. In another application area, the ultrahigh surface area of channel glass is being exploited to create arrays of "microreactor cells" containing immobilized BAC DNAs, for use in repetitive reactions needed for genome mapping and sequencing, including cycle sequencing reactions, PCR, and hybridization mapping of expressed sequences to their genomic clones.

(Supported by SERDP, LDRD)

36. Rapid Screening of DNA Using Maldi-Mass Spectrometry

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Many disease states, both inherited and infectious, can be detected using assays based on the polymerase chain reaction (PCR). These assays amplify (i.e., make many copies of) relatively short segments of DNA that encode disease information in their presence/absence, size, or sequence. For example, an inherited disease could be due to an altered gene for which a PCR assay might produce a product that is smaller than that from the normal gene. Similarly, a PCR assay designed to amplify a targeted DNA region from a bacterium or other agent could be used to detect infectious disease. Once the amplification has been performed, the PCR product must be analyzed. We are developing methods based on matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) as an alternative to existing analysis methods, which include electrophoresis and hybridization techniques. We have demonstrated detection of PCR products with up to 200 bases in a 96-well format and applied the technology to the analysis of PCR products from bacteria, humans, and mice. Advantages of MALDI-MS include speed, accuracy of PCR product size measurement, and the potential for automation. Current areas of study include increasing the useful size range of PCR products that can be

examined, developing rapid methods for purifying and preparing PCR products for MALDI, and collaborating with biologists to tailor PCR and other DNA-based assays for MALDI-MS detection.

(Supported by OBER, NIH)

37. Micromachined Biosensor Arrays

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Calorimetric measurement techniques can directly provide structurally relevant information and can facilitate the functional analysis of proteins. Biological screening applications of this technology are beginning to emerge. We are using silicon micromachining techniques to construct microcalorimetric biosensors. Calorimetric techniques measure the heat changes accompanying chemical reactions and provide for a universal signal transduction mechanism. This approach will enable parallel screening of proteins and allow for greater use of biomolecules as sensor elements because labeling is unnecessary. We have integrated biomolecules, including enzymes and antibodies, with micromachined thermal sensors for calorimetric biosensing of specific analytes. Additionally, suitable methods of protein attachment and instrumentation for arraying have been developed. Future studies will involve the microarraying of proteins, and other biologically significant molecules, onto arrayed calorimetric sensors. Advances in micromachining techniques will allow for the construction of devices with integrated fluidics and sensors that will offer greater use and an economy of scale over conventional devices.

(Supported by LDRD)

38. Medical Telesensor Application-Specific Integrated Circuits (ASICs)

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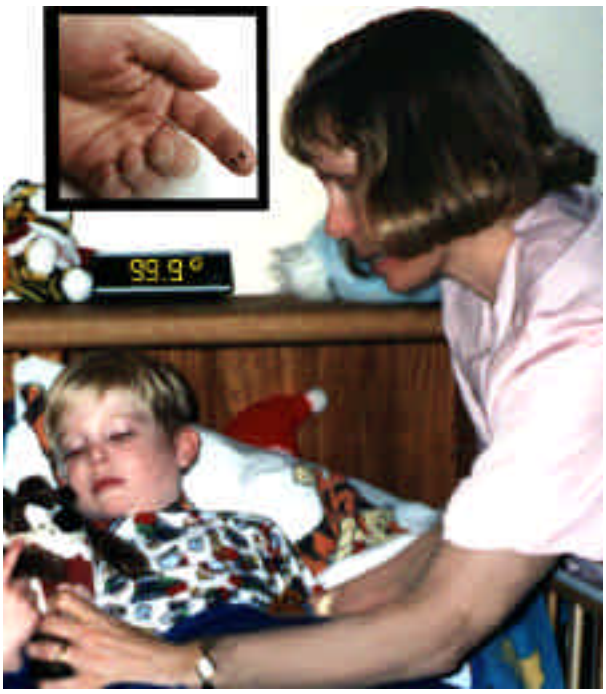

Dr. Thomas Ferrell's Group is developing self-contained wireless telesensor ASICs which each measure one or more vital signs and transmit the data to a nearby intelligent transceiver. The first chip is one for body temperature that can be placed well within the ear canal where it can remain for several months. (See photo of simulation above.) The next chip is a pulse oximeter and this will be followed by a sensor for blood pressure (using pulse analytics). Respiration and skin conductivity would complete the telesensor set. Since spread-spectrum radio transmission is used, the chips can be used in sensitive electronic environments and can be uniquely coded. The antenna, thin-film lithium-ion battery, and stabilization crystal are mounted on a carrier substrate for the temperature telesensor chip and the complete package is approximately 3 mm on a side and 2 to 3 mm thick. The sensor uses the response of mated transistors to the absolute temperature and is

accurate to one-tenth degree Celsius. The duty cycle is typically a few microseconds every 15 minutes. The current form of the pulse oximeter is a small finger ring containing two light-emitting diodes and a photodiode detector as well as the electronics and battery, antenna, and crystal. This system transmits the pulse rate and oxygen saturation. With two pulse-oximeter telesensors it is possible to perform pulse analytics to determine blood pressure. Applications of telesensors include not only inpatient monitoring but also home-based monitoring and monitoring of persons in hazardous environments or in situations requiring extended or high performance. Our chips are made in several stages. Initially, a benchtop system is made and the electronics and sensors are developed as discrete components. The system is then simulated on networked computer workstations for reduction to silicon. The final integrated circuit design is sent to a fabrication foundry and the returned chips are tested and the designs are revised for corrections or for reduced size. The inset in the photograph shows two complete temperature telesensors on the finger. With reduced linewidths these may be reduced in size by a factor of 4. However, even with the use of novel, fractal-based, folded-slot antennas, further reductions require moving from the present 902-928 MHz medical band to the 2 GHz range. This possibility is only now becoming economical.

(Supported by DARPA)

39. Lab-on-a-Chip Technologies for Medical Diagnostics and Drug Discovery

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Lab-on-a-Chip technologies are microfabricated fluidic structures that attempt to perform bench-scale chemical and biochemical procedures under computer control. The long range goal of such devices is to monolithically integrate all of the procedures for a specific experiment or assay. For example, we have demonstrated an integrated device for genetic diagnostics which accepts bacterial cells and performs a multiplex PCR analysis followed by PCR sizing of the amplicons. Another example is an integrated device for performing Restriction Fragment Length Polymorphism (RFLP) experiments. In the latter demonstration, results from subnanoliter samples are produced in five minutes. There are many possible extensions to this work that include all general clinical diagnostic experiments. Moreover, the technology has application to many phases of the drug discovery process, including genomics, proteomics, and high throughput screening of compound libraries against both molecular and cellular targets. Preliminary results have been demonstrated in all of these areas. The general advantages of the technology include automated processing of materials at volumes that are four to six orders of magnitude below the smallest possible at the benchtop with speeds advantages of 10 to 100.

(Supported by LDRD, CRADAs, OBER)

40. Integrated Biochip for Medical Diagnosis

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Rapid, simple, cost-effective medical devices for screening multiple medical diseases and infectious pathogens are essential for early diagnosis and improved treatments of many illnesses. An important factor in medical diagnostics is rapid, selective, and sensitive detection of biochemical substances (proteins, metabolites, nucleic acids), biological species or living systems (bacteria, virus or related components) at ultra-trace levels in biological samples (e.g., tissues, blood and other bodily fluids). To achieve the required level of sensitivity and specificity in detection, it is often necessary to use a biosensor that is capable of identifying and differentiating a large number of biochemical constituents in complex samples. Until now, most DNA biosensors or microchips previously reported use small sample substrates containing microarrays of DNA (often referred to as gene chips), but these systems are based on an external detector, such as a confocal microscope equipped with a CCD for detection. Such systems have a table-top size and are suited for laboratory-oriented applications. The present DNA

biochip offers several advantages in size, performance, fabrication, analysis and production cost due to its integrated optical sensing microchip. The small sizes of the probes (microliter to nanoliter) minimize sample requirement and reduce reagent and waste requirement. Highly integrated systems lead to a reduction in noise and an increase in signal due to the improved efficiency of sample collection and the reduction of interfaces. The capability of large-scale production using low-cost IC technology is an important advantage. The assembly process of various components is made simple by integration of several elements on a single chip. For medical applications, this cost advantage will allow the development of extremely low cost, disposable biochips that can be used for in-home medical diagnostics of diseases without the need of sending samples to a laboratory for analysis. The DNA biochip system offers a unique combination of performance capabilities and analytical features of merit not available in any other DNA analysis system currently available. With its multichannel capability, the DNA biochip technology described in this work is currently the only system that is based on an optical sensing microchip system having integrated an signal amplifier and data treatment on-board. The DNA biochip device allows simultaneous detection of multiple DNA targets simultaneously.

(Supported by OBER)

41. Electronic Nose on a Chip

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Novel sensing techniques, functionally equivalent to a dog's nose, are being developed to sensitively identify a variety of chemical vapors. Detection applications include hidden explosives, drugs, food quality, disease diagnosis, etc. In such a device, combination of vapors would be detected and analyzed in real time by a multiple-input micro-electro-mechanical (MEMS) device.

Microcantilever sensor technology is a next-generation electromechanical technique with broad applications in chemical, physical, and biological detection. The primary advantage of the microcantilever method originates from its sensitivity that is based on the ability to detect cantilever motion with sub-nanometer precision as well as the ease with which it may be fabricated into a multi-element sensor array for real-time analysis under ambient conditions. Both complex mixture analysis and chemical selectivity can be achieved by evaluating the ensemble response of the array. No other portable sensor technology offers such versatility. Two devices based on cantilevers with such unprecedented sensitivity, namely a mercury vapor sensor and an infrared detector, were recognized by the R&D 100 Award in 1996. An initial demonstration of this concept is a one-dimensional, selectively coated, ten-element microcantilever array. Readout is performed using a companion analog readout chip also designed at ORNL specifically for the microcantilever arrays.Ultimately, we plan to coat each element with a different chemical coating, each selectively responding to components of a complex mixture. Key enabling factors of large arrays also include both redundancy and chemical specificity provided by an *ensemble* response of the array. Thus, selectivity need not be limited to that which can be accomplished by individual sensors. Large-scale manufacturing of integrated devices will drive the

MEMS-based sensor-array prototype with wireless reporting, powered by four AA batteries.

cost of such devices to enable point-of-care and even consumer applications.

(Supported by OBER, CRADA)

42. Boron Neutron Capture Therapy (BNCT) Real Time Dosimetry

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Epithermal/thermal boron neutron capture therapy (BNCT) is a treatment method for malignant tumors. Because the doses and dose rates for medical therapeutic radiation are very close to the normal tissue tolerance, small errors in radiation delivery can result in ineffective treatment or overdose. A substantial need exists for a device that will monitor, in real time, the radiation dose delivered to a patient. PNNL has developed a scintillating glass optical fiber that is sensitive to thermal neutrons. The small size of the fibers (150-m diameter) offers the possibility of body surface or in vivo flux monitoring at multiple points within the radiation field. The count rate of such detectors can be as high as 10 MHz because the lifetime of the cerium activator is fast (60 ns). In collaboration with INEL's BNCT team, preliminary experiments have been performed. Fluxes typical of those in BNCT (i.e., 10^{10} n/cm^2/sec) have been measured with a single fiber. Under this program development efforts were conducted to bring the dosimeter from a laboratory prototype to a device useful in the BNCT research program and, ultimately in the treatment of patients.

(Supported by DOE OBER)

43. Accelerated Molecular Discovery Arrays

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This new Grand Challenge LDRD, termed "Accelerated Molecular Discovery Arrays" is focused on the discovery of signal molecules—those molecules (small molecules, peptides, and proteins) which turn on and off various body responses (hunger, sleep, fear, cell growth, disease, ...). We have begun work on this project in collaboration with Peter Schultz and Jean Frechet (UCB and LBNL). Signal molecules are secreted locally (inter-cellular) and at very low levels (< nM) amidst a sea (thousands) of cellular proteins. Thus the first order problem is a highly sensitive analytical technique that can rapidly identify any changes in the thousands of cellular molecules upon exposure to selected stimuli. The microchromatography techniques we have been developing for MicroChemLab are ideally suited for this. We will expand the MicroChemLab technology to develop arrays of highly parallel separation channels followed by ultrasensitive laser-induced fluorescence detection. In the first year, we will establish the amount of separation diversity needed and decide on the combination of parallel and tandem separations and stationary phases that will provide that diversity. In year 2, we will fabricate that system and verify against a known signal molecule, and in year 3 we will start the discovery process. The discovery of signal molecules—the "on/off" switches in the body—would enable a major new biological paradigm centered on "mining the body." This paradigm would allow us to take advantage of the body's existing armies of proteins and action pathways, by identifying the "switches to turn these on and off." The discovery and identification of signal molecules will accelerate biomedical understanding and will open up new therapies based on signal molecules and their regulation.

(Supported by Laboratory Directed Research and Development)

44. Measuring Blood Rheology Using Thickness-Shear-Mode Resonators

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A thickness-shear-mode (TSM) resonator, consisting of a thin piezoelectric (AT-cut quartz) crystal with metal electrodes on the two faces, measures the viscous properties of surface contacting materials. For Newtonian fluids on the crystal surface, the resonant frequency shift and change in oscillation magnitude are both proportional to the density–viscosity of the fluid. For non-Newtonian fluids and viscoelastic solids, the frequency shift and change in magnitude vary in differing proportions that allow extraction of static viscosity, molecular reorientation times, density, and elastic moduli. Adaptation of the TSM resonator technology for clinical applications addresses some of the unique features of blood and overcomes many of the difficulties in its rheological characterization: (1) Blood is non-Newtonian. The plasma is Newtonian, but the red blood cells, which compose about 35-40% of the volume, are deformable and contribute to shear rate dependence and viscoelastic behavior. The TSM resonator can determine both Newtonian and non-Newtonian properties. (2) Sample volumes available from patients are small and must be analyzed quickly as they change rapidly over time. Only a few microliters of blood are needed for complete characterization by the TSM resonators, and real-time measurements are made using in-line flow cells and, eventually, *in vivo* placement. These investigations are performed in collaboration with Prof. William Lee at the University of South Florida (USF) and Dr. Electra Gizeli at Cambridge University. Prof. Lee specializes in hemorheology and possesses the laboratory environment needed for clinical trials and prototype demonstrations. Dr. Gizeli is an expert in material biocompatibility and is developing interface films that eliminate undesirable non-specific binding and enable direct detection of blood properties. Blood rheology is a focused area of general rheology and has significant interest for clinical applications. A number of disease processes are known to impact blood rheology: many forms of cancer, diabetes, chronic anxiety, and cardiovascular disease. Clinical therapies, such as the intravenous introduction of dextran solutions, hemodilation procedures employed by cardiologists, and use of pharmaceuticals for vasodilation, significantly influence blood rheology. Additionally, hyperviscosity leads to lower blood flow rates; red blood cell aggregation; decreases in oxygen transport, tissue nourishment rate, and waste removal rate; increases in clot formation and bacterial/viral attack; and a disruption in osmotic status.

(Supported by Laboratory Directed Research and Development, NIH)

45. Biological Weapon Detector Using Bioaffinity Array Impedance Analysis with Chemical Amplification Through RedOx Recycling - BioCCD

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A novel structure for ultrasensitive detection (1 particle per liter of air) of BW agents, e.g., anthrax, will be developed to the sensor prototype stage. Surface-attached specific affinity components (antibodies, combinatorial peptides, or glycolipids) will capture a particle on one element of a microelectrode array monitored via impedance analysis transduction, chemically amplified through disruption of redox recycling. Faradaic impedance is monitored using a reversible redox couple ([e.g., Fe(CN)6]4-/3-) recycled between its oxidation states by each microelectrode and a shared counter electrode. Capture of a single pathogen blocks one microelectrode, disrupting the redox cycle at that electrode. An array of pathogen-sized, individually monitored, small-area (2×2 μm^2) microelectrodes enables single-particle detection. Many identically-coated array elements (100's - 1000) covering one surface of the flow cell enhances the probability of single-particle capture and provides interferant discrimination by spatio-temporal tracking of the capture/release of each particle traversing the cell. Small capture area is key to single particle detection; a multi-element array is key to rapid response, realistic sample volume, and improved discrimination. A 20 element

microelectrode array has been designed and fabricated on a silicon substrate. All leads are protected by glass and silicon nitride except the 4 micron diameter Pt active areas and the counterelectrodes. A proof of principle has been made using 4 micron latex spheres as surrogates for the bioparticles. The electrochemical current shows a huge moduation with the position of the sphere in the hole. The analogy would be the flapper in a toilet tank: when the flapper is off the water (electrochemical current) flows freely through the hole. When the flapper drops in place, the flow stops. If the flapper is not a perfect match to the rim of the hole, some leakage occurs, but it is easy to tell if the flapper is in place. This encouraging result means that we will go forward to solve the other formidable problems in this proposed technology: read-out of hundreds or thousands of pixels, selecting bioaffinity coatings that give effective screening, fluidic handling of the bioparticles, etc. While this technology was conceived as a way of identifying pathogens like anthrax, there is no reason why it could not be used to screen for other interesting bioparticles, both pathogens and beneficial bugs. Widespread uses in medicine, and even home health care and food safety, can be envisaged.

(Supported by Laboratory Directed Research and Development)

46. Combinatorial BioFET Microsensor Arrays

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Chemical agent detector based upon enzymatic recognition of chemical warfare agents and electrochemical signal from enzymatic cleavage of chemical warfare agents. Unique to the sensor strategy is using an array of genetically engineered enzymes to provide a signature response to various analytes. The principal result is the detection of organophosphate pesticide chemical warfare surrogate compounds at one micromolar with less than 30 second device response time. A unique strategy for patterning multiple biological recognition elements on a single surface was developed. This approach lends itself to manufacturing large numbers of components with immobilized biological recognition units since it uses established semiconductor processing tools. Wafer scale immobilization and patterning of biological receptors is key to achieving the economies of scale required to make production of devices with specialized bioreceptors a profitable undertaking.

[Supported by Laboratory Directed Research and Development (FY96–FY97), DOE NN-20 (FY98 and ongoing)]

47. Non-Invasive Biomedical Monitoring

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Using near-infrared spectroscopy and chemometric methods it is possible to measure various blood analytes such as glucose, alcohol, urea, pH, etc. noninvasively with clinically relevant accuracy and precision. The body of work has included patents (6 U.S. patents held or co-held by Sandia) and papers on the non-invasive monitoring of glucose in diabetics, non-invasive monitoring of blood gases in critically ill patients, non-invasive monitoring of oxygenation of the blood of human fetuses during labor and delivery, and the in-vitro and in-vivo noninvasive detection of cancer cells. The American Diabetes Association (ADA) has estimated that 14 million Americans, or approximately 6% of the nation's population, have diabetic conditions. Diabetes mellitus is the seventh leading cause of death in the United States. The overall economic burden of diabetes in the United States exceeds $40 billion each year. Diabetes is responsible for 12,000 cases of blindness and 10,000 cases of end stage renal disease each year. It is likely that these serious complications, the associated morbidity, and the cost of care can be greatly reduced by improving control of blood glucose levels. Current widely used methods for determining blood glucose levels require the diabetic to draw a blood sample for chemical analysis. Such a method entails the painful lancing or invasion of the skin to obtain a drop of blood. Most diabetics find this procedure to be painful, inconvenient, costly, and often a deterrent to regular glucose testing. A noninvasive blood analyte monitor would provide virtually continuous glucose measurements without the pain or inconvenience of the finger stick

method while providing more reliable and consistent analytical results. Physicians believe that far better therapy and control of blood glucose levels for the diabetic can be achieved through a glucose monitoring program that encourages rather than deters glucose testing. Arterial blood gas parameters are vital measurements for the critical care or trauma patient, providing the cornerstone for diagnosis and management of cardiopulmonary disease in the critical care patient. Current methods of arterial blood gas measurement are painful, costly, and potentially infectious. These measurements are typically the most frequently ordered test in a hospital intensive care unit. Industry sources estimate that approximately 40 million discrete laboratory analyses for blood gases are performed annually in the United States. Each arterial blood draw must be transported from the patient to the laboratory, requiring significant time during which patient status can change. Non-invasive blood gas monitoring has the potential to provide the physician with near-real-time feedback to interactively manage patient treatment at the bedside. The potential impact of non-invasive monitoring of other medically important factors is expected to be analogous. Time frame: FY88 and ongoing.

[Supported by Rio Grande Medical Technologies (RGMT)–exclusive licensee; the blood gas work was previously supported by the DARPA TRP program]

48. Investigation of Technologies to Improve Fiber Optic Biosensors for Counter-Proliferation Purposes

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The performance of fluorometric biosensors, with chromophoric dyes to stain biologically specific molecules, such as antibodies and proteins, to detect and quantify biologically significant processes and reactions is presented. The principal result is that fiber-optic molecular sensors can provide an effective method for the quantitative detection of biological agents that may be used as weapons of mass destruction. The combination of time resolved laser excitation of fluorescence and synchronous heterodyne detection is superior to other methods for compact bimolecular sensing using fiber optic components. The application of frequency domain fluorescent spectroscopy using coherent communication linked signal processing methods is demonstrated using diode laser excitation and synchronous emission detection methods. Fiber-optic molecular sensors can provide an effective method for the quantitative detection of biological agents that may be used as weapons of mass destruction. A compact all solid state fiber-optic biosensor probe can be constructed. Practical implementation of such a probe could be undertaken using currently available optical components and hybrid integrated circuits used in wireless telecommunications equipment. These compact all solid state probes could be used to detect a variety of toxic agents throughout a network of fiber-optic sensors as a defense against chemical and biological attacks. Less complex versions could be used to monitor the biological contamination from infectious pathogens to agricultural chemicals, such as pesticides and herbicides. Time frame: FY96–FY97.

(Supported by Laboratory Directed Research and Development)

49. Parallel Microseparations-Based Detection of Biological Toxins

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We are currently developing a compact, portable, low-power detection system for biological toxins at low (e.g., 10^{-12} Molar in solution) concentrations. This device aims to achieve an extremely low false-alarm rate by performing multiple separations on each sample, each yielding independent information about the properties of each substance in the sample. Each toxin will have a "fingerprint" of characteristics that uniquely identify it, and the system will only identify a particular target substance (i.e., toxin) as being present if there is present a compound that fits the exact fingerprint of molecular properties of the target substance. Although the microseparations have been shown to be effective for a variety of small molecules, many toxins are proteins, and much of the capability of our system is being targeted toward the accurate characterization and identification of proteins, as well as small biomoelcules.

Because pathogenic agents are also constituted of biomolecules, and the principal component of many pathogens, such as viruses, is also a unique protein or set of proteins, this technology may also be applicable to detection and identification of pathogens. The strategy of using parallel and sequential separations to identify proteins and biomolecules in analytical biochemistry is not new—in fact it has historically been the principal means by which trace quantities of biologicals have been purified from complex backgrounds and identified. Techniques such as size exclusion (gel filtration), ion-exchange, affinity and reverse-phase chromatography, as well as electrophoresis, allow proteins, peptides, etc., to be separated on the basis of the following properties: mass, acidity/basicity, ligand binding, hydrophobicity, and charge/mass ratio as a function of pH. The significance of our approach is that we have the technology to implement these methods as microseparations that can be run in a chip format, in combination with reactive fluorogenic labeling and extremely sensitive laser-induced fluorescence (LIF) detection based on compact, low-power lasers and detectors. We are currently implementing electrokinetic microseparations for proteins and toxins in capillaries and on chips and designing a complete compact portable detector, including multichannel LIF, data acquisition and analysis, and power supplies. This detector technology, which draws on, but goes beyond the capabilities of SNL's MicroChemLab project, will also have diverse applications in medical diagnostics and biomedical research. Aside from toxicological uses, applications such as rapid, ultrasensitive serum protein profiling should be possible (e.g., instant monitoring of serum lipoproteins) Time frame: FY 97 and ongoing.

(Supported by DOE NN/Chemical and Biological Non-Proliferation Program)

50. Miniature UV Fluorescence Based Biological Agent Sensors

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This project will develop a miniaturized ultraviolet (UV) fluorescence biosensor based on UV-LED (Light Emitting Diode) and miniature spectrometer technology being developed at Sandia. We will evaluate the performance of a LED operating at 360 nm in bio-fluorescence measurements. The LED is based on the AlGaN family which has direct optical band gaps from 364 nm (GaN) to 200 nm (AIN). The wide wavelength range of these materials makes them attractive as compact UV light sources. To date, efforts in developing nitride-based emitters have focused mostly on visible (blue and green) light emission using InGaN as the active region; UV applications using GaN and AlGaN have remained relatively unexplored. The UV LED will be fabricated at Sandia in a recently established MOCVD facility which has demonstrated the growth of GaN materials with structural, optical, and electrical quality comparable to the best reported thus far. The LDRD will 1) develop novel UV LED light sources, 2) demonstrate their usefulness for the detection of bio materials, 3) develop a miniature spectrometer for bio-fluorescence measurements, and 4) culminate with the design for a prototype instrument which can be used for demonstration measurements. The development and demonstration of this miniature biosensor will enable the development of miniature, low power, rugged, point sensor systems for the detection of biological warfare agents in battlefield and counter-terrorism applications. Time frame: FY99 – ongoing.

(Supported by Laboratory Directed Research and Development)

51. Optical Detection of Biologicals

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This project develops a point and standoff detector of biologicals and chemicals for potentially a very wide impact in the fields of battlefield sensing, hospital sterilization, and food safety. A small portable sensor for optical detection of biologicals for healthcare safety, especially sterilization facilities, and a rapid optical detector for use by healthcare workers to detect both live and dead bacteria will be developed. Time frame: FY98–FY99.

[Supported by Kimberly-Clark Corporation (CRADA)]

52. Optical Detection of Pharmaceuticals in Optically Dense Media

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A sensor for the optical detection of chemicals and biologicals of the pharmaceuticals in realistic optical backgrounds such as blood and body fluids will be developed. Detection and concentration estimates for certain classes of chemicals (or pharmaceuticals) and biologicals (i.e., those with sufficient fluorescence cross section) were demonstrated in an optically dense matrix (blood plasma). In research labs this technique may enable the pharmacokinetic studies of pharmaceuticals and poisons. It may also provide a diagnostic tool for clinical use. Time frame: FY97–FY99.

(Supported by Sandia Royalty Funds)

53. Optical Detection of PrpSc

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Development of a sensor for the optical detection of the proteins (prions or PrpSc) associated with transmissible neurodegenetive diseases such as Creutzfeldt-Jakob (CJD) and bovine spongiform encephalopathy (Mad Cow). The fluorescence cross section of PrPSc was calculated from calibrated measurements, for the first time. Discrimination between different forms of PrPSc was performed by multi-spectral fluorescence spectroscopy, demonstrating the feasibility of the method as a means of optical detection. The potential reduction in the time to detect these diseases will decrease from months and years to seconds. Method may be applicable to a wide variety of related diseases and pathogens. Time frame: FY97–FY99

(Supported by Sandia Royalty Funds)

Clinical Measurements

This section lists research projects dealing with a variety of analytic measurement techniques for measurement of biologically important molecules and drugs. The techniques range from variations of standard mass spectrometry to electrospray processes and pulse radiolysis techniques.

54. Technique Measures DNA Damage from Carcinogen

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Small combined fluorescence line-narrowing spectroscopy (FLNS) technique with capillary electrophoresis (CE), a widely used analytical chemistry method. The CE-FLNS technique has been used to identify by-products in urine that result from the reaction between cancer-producing pollutants, such as those found in cigarette smoke, and cellular DNA. The identification of these by-products, called DNA adducts, is important to understanding the first step of a cancer—the chemical attack of carcinogens on DNA. While the CE-FLNS technique has only been used to study cancer from chemicals so far, Small expects it will be used in other areas of biological research, as well as forensic science. This technology has been recognized with an R&D 100 Award in 1998.

(Supported by OBER)

55. Analytical Techniques Measure Trace Components in Cells

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Yeung and colleagues have successfully determined femtomole (10^{-15}) levels of sodium, potassium, and glutathione in single cells and then achieved the separation and fluorescence measurement of the major proteins in single erythrocytes at the 10^{-18} mole level. The group has also developed an assay that permits detection down to 800 molecules (10^{-21} moles) of the enzyme lactate dehydrogenase (LDH) in single human erythrocytes by means of an on-column reaction and capillary electrophoresis. The five major forms of the isoenzyme can be quantified, a finding that may shed light on cellular functions, since LDH isoenzyme distributions have been correlated with various forms of cancer.

(Supported by BES)

56. Biophysics of Myeloma Pathology

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A neoplastic proliferation of antibody-producing cells in the cancer, multiple myeloma, leads to a monoclonal overproduction of antibodies and antibody light chains. These proteins generate substantial disease complications in approximately half of the myeloma patients and are often the direct cause of death. Focused study of these proteins enabled

researchers to achieve the initial crystallographic and amino acid sequencing studies that provided insight into the structural and genetic origins of the immune system. These disease processes result from self-assembly or aggregation of the monoclonal light chain leading to precipitation and deposition. In some cases, deposition occurs primarily in a single organ; in others it is dispersed systemically throughout the body. Deposition can occur as highly structured amyloid fibrils non-fibrillar punctate deposits (resulting in light chain deposition disease) or amorphously in kidney tubules (resulting in tubular cast nephropathy). Some patients experience no problems, despite production rates as high as 100 g per day. These phenomena can be described as conformational diseases that result from the consequences of physicochemical properties of the protein that lead to dysfunctional expression. Other examples of conformational diseases include several forms of amyloidosis resulting from other proteins, including the beta-peptide associated with Alzheimer's disease, the prion diseases (mad cow disease, kuru), cataracts, and sickle cell anemia. Light-chain diseases provide a robust model system for conformational disease in that several different manifestations arise from the same tertiary structure. However, unlike sickle cell hemoglobin in which a single amino acid variation distinguishes normal from pathogenic forms, understanding antibody light chains is a far more difficult challenge. To date, researchers have not found two identical light chains in different patients. Variations range from 10% to 60% as a consequence of numerous germ line genes for light chains and from the accumulation of somatic mutations during the normal course of immune system diversification. No signature of structural variation emerged from early primary structure determinations and it was not clear if the protein is the principle causal factor of the disease process or if it originates from patient-specific variations. Argonne's early work, prompted by clinical collaborators at the University of Tennessee Medical Center, demonstrates that proteins associated with patient pathology exhibit a tendency to aggregate when tested by size-exclusion chromatography. This finding substantially links the protein as the primary agent of pathology. Subsequent collaborations include groups at New York University and Dekker Hospital, Paris. Current studies focus on detailed analysis of primary structure data and the use of site-specific mutational and crystallographic analyses to address the cryptic role of amino acid variations. The Laboratory has assembled a database (exceeding three hundred entries) of light chains produced by patients with B-cell diseases. These data, combined with approximately 2000 "normal" human sequences, and an equal number of mouse sequences, provide an immense "bioinformatic" challenge. These data may constitute the largest set of diverse sequences of proteins, all of which have highly homologous three-dimensional structures, but diverse physicochemical properties. Argonne's findings prove that certain variations of amino acids are somewhat overrepresented in the pathological light chains. When tested in recombinant forms, the pathological light chains are less stable than their nonpathological counterparts. Site-specific mutation studies indicate that the amino acid variations can be categorized in three sets. Approximately one-third of the variations have little effect, while one-third are destabilizing. Surprisingly, a substantial number of variations are stabilizing. Research shows that a rather sharp stability threshold distinguishes light chains that form fibrils (*in vitro*) from those that do not. Thus, the pathogenic potential of these proteins results from the cumulative effects of several variations; the occurrence or absence of any one of several can change the outcome. As primary structure data accumulates, primarily from "Bence Jones proteins," certain mutations are absent upon observing the light chains obtained from the urine of myeloma patients. Argonne predicted and confirmed that these "missing" mutations would be observed in proteins for which data were obtained from deposited material extracted from tissues and from cDNA obtained from B-cells. Through collaboration with a University of Chicago group which studies the role of a chaperone (BiP) in the intracellular assembly of antibodies, Argonne finds that certain light chains, containing mutations assumed to be incompatible with a properly folded chain, are introduced into functional antibodies. Fab structures provide additional examples of this phenomenon. Thus, interaction with the heavy chain stabilizes the light chain (and vice versa) and the heavy chain becomes a major factor in the existence of certain light chain diseases. Without the accessory stabilization, the host B-cell which produces a potentially pathogenic light chain does not produce a functional antibody and is eliminated by a normal apoptotic mechanism, prior to its eventually neoplastic transformation leading to the light chain disease. In collaborative studies directed to identifying potential BiP binding sites, Laboratory researchers find that light chain-related peptides serve as substrates for the chaperone. Recently, Argonne results show that one of these peptides blocks fibril formation in an *in vitro* assay. While

this peptide may serve directly as a starting point for the development of drugs useful to prevent or reverse the currently untreatable production and deposition of amyloid, it will also serve as a new tool for determination of the mechanism of fibril assembly. The mode of action of the peptide may be to insert itself into the site normally occupied by its cognate counterpart in the intact protein, possibly blocking a site involved in inter-subunit interactions in the fibril. If so, the implications of this observation may extend well beyond light chain diseases and provide a new strategic approach in the treatment of many conformational diseases. Note: This project is in its sixth year of NIH funding.

(Supported by NIH, National Institutes of Diabetes and Digestive and Kidney Diseases)

57. Motor Neuron Diseases

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Wasted mice bear an autosomal recessive gene (*wst*) that causes motor neuron degeneration, radiation sensitivity in T lymphocytes, and immunodeficiency. Recent Argonne research has demonstrated a 3-bp (3G) deletional mutation in the promoter (5' region) of the PCNA gene of wasted mice that is not found in healthy control littermates. Researchers are testing the hypothesis that abnormal regulation of PCNA expression caused by this 3G deletion is responsible for the *wst* mutation and the wasted phenotype of these mice. Specifically, studies will confirm the 3G deletion as the molecular basis for the motor neuron disease in wasted mice through the use of vector-based and transgenic approaches; identify the protein(s) and their gene(s) that bind specifically to the 11G element in the PCNA promoter, mapping the protein binding regions, and determine the mechanisms underlying motor neuron-specific function; investigate the reasons for motor neuron death in wasted mice via studies of tissues from wasted mice in terms of pathology and apoptosis, in vitro studies of neuronal cells from wasted mice (for PCNA expression, apoptosis, and oxidative damage, studies examining a possible common pathway of motor neuronal cell death in mice, and differential display of wasted and FALS transgenic mouse cDNA; sequence PCNA promoters in ALS, FALS, and other motor neuron diseases, identifying possible mutations associated with the diseases, and analyze these disorders for sensitivity to oxidative stress and PCNA expression. In so doing, these experiments will test aspects of the following model: The absence of PCNA expression in thymus and motor neurons of wasted mice causes cellular apoptosis. This absence of expression is mediated by a positive transfactor which can bind to the wild-type but not the wasted-mutant PCNA promoter. The bound protein induces late expression of PCNA in motor neurons and T lymphocytes. ALS and FALS patients have abnormalities in this pathway either via a common SOD/PCNA circuit or via differing SOD and PCNA pathways that result in apoptosis and cell death. Invention reports have been submitted. This research is likely to provide technology and methodology useful in diagnosing and treating Anterior Lateral Sclerosis and other motor neuron diseases.

[Supported by University of Chicago (NIH)]

58. Development of Diagnostics for Lyme Borreliosis

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The principal goal of this project is to use molecular and immunological techniques to develop more sensitive and specific tests for the diagnosis of Lyme borreliosis. In this project we will use specimens of well-characterized patients with Lyme disease. We have developed a network in which specimens are prospectively and longitudinally collected from patients in whom detailed case report forms are available. The diagnostic tests which we will develop can be categorized into two groups: (1) To use recombinant proteins and peptides to characterize the humoral response to this pathogen and to utilize these recombinant proteins to develop antibody screening assays and (2) to utilize molecular and immunological techniques to identify the pathogen or its antigenic products in patient specimens. The Outer Surface Protein (Osp) A, p41, p66, p73, and p93 antigens have been cloned. The epitopes of OspA and p41 have been mapped. Epitopes of p66 are to be mapped under this program. Sequencing of the genes

encoding p93 and p73 will be completed, and the epitopes will be mapped. In addition, we will continue to define the humoral response to the OspA antigen which is associated with the development of chronic disease. Specific immunoassays will be developed from cloned antigens.

(Supported by State University of NY, Stony Brook)

59. Methyl Histadine Kinetics as an Indicator of Muscle Mass and Metabolism

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Maintenance of muscle mass is a critical problem in disease, in old age and in any program intended to reduce fat mass. The problem is further complicated in that there are no effective methods available to directly measure muscle mass or muscle breakdown. Thus the need for intervention in disease and a method of effective intervention is left primarily to subjective methods. The proposed studies are aimed at developing a basic concept of protein biochemistry into a diagnostic tool. This tool will be used to measure muscle mass and metabolism in normal and disease states. The principle to be developed involves the kinetic modeling of an amino acid unique to muscle protein, 3-methylhistidine. Using a stable (nonradioactive) isotope of this amino acid it is possible to measure the flux in the blood and tissues of humans by only sampling blood. This "decay" of isotope in blood can then be mathematically described by a compartmental model which can, in turn, predict the rate of muscle protein turnover. The parameters, mass of compartments, and flux between compartments of the model can be used to predict muscle mass. Phase I of this project will provide direct evidence of the relationship between the 3-methylhistidine compartmental model and muscle mass. Muscle and nonmuscle mass will be estimated independently from equations that utilize total body nitrogen (total-body neutron activation) and total body potassium. Phase II of the project will be focusing on the development of a commercially available test which is economical and easy to use. It is envisioned that such a test could be used in a variety of clinical disease states (cancer, AIDS and dystrophic diseases) and to monitor muscle changes in diet programs intended to maximize fat loss and minimize muscle loss.

(Supported by Metabolic Technologies, Inc.)

60. Analysis of Biological Fluids by Ion Mobility Spectrometry

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Research is about to begin at the INEEL which uses highly sensitive analytical instrumentation originally developed for the detection of chemical weapons and explosives and focuses on medical diagnostics. Ion mobility spectrometry is a very sensitive, rugged and portable technique which is currently being applied to the study of biological fluids at the INEEL. Specific medical and veterinary applications have been conceptualized, and the funding to pursue this work is being sought. It is envisioned that the actual evaluation of the technique for medical diagnostics will take place in the next 18 months, most likely starting on animal diseases in conjunction with a veterinary school, and then transitioning into human diseases in conjunction with a medical school. It is envisioned that the successful application of this instrumentation will result in very high speed medical screening which can reduce the need for extensive exploratory medical testing on patients, thus greatly reducing costs. Also, the relatively low cost and portability of the instrument can lead to greater use in the community, which can raise the country's overall health level by providing low cost, high throughput disease screening clinics.

(Supported by Industrial Company)

61. SIMS Technology for the Study of Microsurface Chemistry

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This program is developing an imaging secondary ion Mass Spectrometer (SIMS) for the purpose of analyzing the molecular composition of the materials on a surface as a function of position. There are commercial instruments on the market for accomplishing this, but they are limited in their ability to analyze the surfaces of electrically insulating materials and in their ability to provide molecular (as opposed to atomic) information. Technology has been developed at the INEEL specifically for addressing these issues. Instruments for the analysis of broad areas of insulating materials have been analyzed very successfully, and these techniques are now being applied in the development of an imaging instrument. There are a wide range of potential applications, ranging from interaction chemistries of chemicals with minerals, to the analysis of biological and medical samples.

(Supported by DOE/OBER)

62. Microbiological Identification from Cell Membrane

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Rapid, microorganism identification remains a difficult problem, despite its importance in biological warfare scenarios, terrorist interdiction, environments contaminated with pathogenic microorganisms, and environmental remediation. The extreme need for techniques which can quickly identify microorganisms on a wide variety of samples types has not yet been satisfied by any instrumentation currently on the market. Since traditional microbiological detection has not fulfilled the requirements for rapid microorganism identification, a less traditional approach must be developed. We propose to utilize the novel technique, ion trap static secondary ion mass spectrometry (IT-SIMS), which is being developed at the INEEL, for the detection of microorganisms on environmental samples (such as mineral and vegetation surfaces).

[Supported by Internal funds (LDRD)]

63. Noninvasive Measurement of Drug Concentrations in Tissue

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We have developed an optical instrument and method for measuring in real time, and with site-specificity, the absolute concentrations of drugs or other compounds in tissue. New computational methods allow determination of the dependence of the pathlengths of photons in tissue on the tissue scattering properties, so that analysis of the optical spectra enables determination of the drug concentrations. We have tested the method by measuring the pharmacokinetics of two chemotherapy agents in live-animal tumor models. The fiber-optic system was used to perform pharmacokinetic measurements on the tumors following drug administration. Time histories of the drug concentrations in the tumors agreed with the known pharmacokinetics of the two drugs, and HPLC assays following sacrifice showed linear correlation with optical values. Most photodynamic therapy agents and many chemotherapy drugs are good candidates for this method.

(Support awaiting disposition of a proposal to NIH)

64. Low Frequency Impedance Spectroscopy for Biomolecular Characterizations

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Low frequency impedance spectroscopy has been developed for the characterization of amino acids and proteins in collaboration with the UNESCO Center for Membrane Science and Technology at the

University of NSW in Sydney Australia. Impedance spectroscopy measures the electrical characteristics of biomolecules as a function of frequency from 0.0001mHertz to 1000 000 Hz. It is a nondestructive measurement and uses a sensitive four terminal computer controlled measuring device. A very small electric charge is introduced into the specimen and the behavior of these charges within the sample is measured as a function of frequency to reveal the electrical characteristics of the sample's constituents. These measurements are ultra sensitive to the electrical interactions of substrates (drugs, vitamins, etc.) with the biochemical machinery. This technology is in its infancy but is expected to have a profound effect in the future, since it can be used to characterize membranes from the physiological measurements of cholesterol interactions in humans to the development of synthetic membranes for industrial waste separation and desalination.

[Supported by DOE (OBER)]

65. Beryllium Health Effects

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The Beryllium Health Effects Program has two main goals: 1) To develop improved methods for detecting beryllium sensitivity and applying these improved methods to the testing of LANL beryllium workers as part of beryllium medical surveillance; and 2) identifying genetic markers associated with increased genetic risk for developing Chronic Beryllium Disease (CBD). We have developed a flow cytometry assay that combines blood lymphocyte immunophenotyping with cell cycle analysis. The result is a very specific test for lymphocyte subset growth in response to beryllium in vitro. CD4 cells appear to be the "bad actors" in individuals with beryllium sensitivity and CBD. The assay is being used to screen individual beryllium workers for true beryllium sensitivity, at an early stage of CBD. In the genetic susceptibility study we are using direct DNA sequencing to identify particular HLA alleles that are more highly associated with CBD. We have found that certain HLA-DPB1 and HLAPDA1 alleles have high association with CBD, and we hypothesize that the presence and copy number of these particular alleles is a factor that confers greater risk for development of CBD. A microsphere based flow cytometry assay has been developed for rapidly typing individuals for specific CBD-associated HLA alleles.

(Supported by DOE, Office of Biological and Environmental Research and the Office of Environmental Safety and Health)

66. Monitoring Inflammatory Cytokines Using Maldi Mass Spectrometry

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This project addresses the need for rapid analyses of mouse body fluids for proteins which are produced directly or indirectly due to the aberrant expression of genes in mutant animals. We are developing procedures to use matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) for the rapid analyses of cytokines which are markers for malfunctions in the immune system's inflammatory pathways. Specific antibodies or receptors produced from recombinant DNA have been used to capture and concentrate target cytokines which can subsequently be detected using MALDI-MS. The capture of the protein is done on microbeads that can be collected by centrifugation or in a magnetic field and then can be transferred by microfluidics pipetting to an appropriate mass spectrometry sample plate for automated analysis. Although this project focuses on cytokines, the technique is adaptable to analysis of any moderately sized protein. Cytokines have been chosen since they play a central role in the body's response. Aberrant immune recognition leads to the recruitment of cells which produce cytokines and can result in chronic disease. It is likely that inflammation in mutant mice will be detected as a secondary reaction to the primary mutational defect. The inflammation event(s) can occur early in life and slowly alter metabolic and repair pathways that ultimately lead to disease. Detection of aberrant cytokine expression in juvenile animals should be an early marker for animals destined to develop chronic inflammatory conditions (inflammatory bowel disease, arthritis, etc.). These tests should detect differences in levels of expression and/or post-translational modification of cytokines in different strains of

animals in a screening set. The measurements of specific cytokines in a defined mutant strain would also be valuable as a means to define the mechanisms leading to the mutant phenotype. This developing technology establishes ORNL as a state-of-the-art institution in analyses of protein expression. Specific, automated detection of proteins such as cytokines is a significant addition to Dr. Dabney Johnson's phenotype screening "screen-o-type" center and contributes to work proposed for embryonic stem cell analyses (Drs. Ed Michaud and Gene Rinchik).

(Supported by LDRD, OBER)

67. Electrospray Ionization and Ion/Ion Chemistry for Rapid ID of Pathogens

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Instrumentation and methodologies based on electrospray ionization and tandem mass spectrometry are being developed for the rapid detection and identification of proteins indicative of bio-toxins, viruses, and bacteria. The approach involves subjecting relatively complex protein mixtures derived from, for example, cells, sera, environmental wipe samples, etc. to electrospray ionization. The ions derived therefrom are then subjected to ion/ion reactions to simplify interpretation of the complex data normally obtained from electrospray of protein mixtures. Proteins indicative of pathogens or disease states can be identified by dissociating the proteins and either matching the spectrum of dissociation products to a library spectrum or matching a sequence tag to a protein database. The overall technology promises to eliminate time-consuming gel electrophoresis and chromatography steps thereby allowing for high throughput measurements. This approach has relevance to public health, medical diagnosis, and disease prevention.

(Supported by BES, NIH)

68. New Approaches for Monitoring of Trace Compounds in Physiological Media

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Current techniques for monitoring exposure or dosimetry require extraction of the target compounds from blood, serum, urine, or other physiological media, concentration of the extract, and then analysis by chromatographic techniques coupled by mass spectrometry. These assays are expensive and require hours for each analysis. We are developing new approaches to detect targeted compounds at the part-per-billion (and lower) level by taking advantage of the electrochemical behavior of the electrospray process that is used in conjunction with mass spectrometry. Target compounds from the sample (i.e., urine with no sample pretreatment) are first electrochemically deposited on an electrode. The electrode surface is washed to remove matrix impurities, and then the target compound is electrochemically stripped from the electrode and introduced into the mass spectrometry via electrospray ionization. This process is fully automated using a computer-driven switching valve device. The total analysis time is reduced to a few minutes, rather than hours. In addition, the sample size may be reduced to a few microliters volume. We have demonstrated this technique on metals in urine (e.g., thallium detected at parts-per-quadrillion levels) and several drugs and drug metabolites (e.g. tamoxifen detected at <1 part per billion in urine). The technique is being developed to allow rapid analysis of target compounds in small (minimally invasive) sample volumes to facilitate monitoring of target compounds for exposure, dosimetry, and epidemiological studies.

(Supported by BES, NIH)

69. Utility of Breath Analysis for Noninvasive Monitoring of Infectious Disease: Focus on Burn Victims

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The objective of this project is to test the hypothesis that the inflammatory cascade triggered by severe burn trauma and subsequent systemic bacterial infection produces a predictable evolution of volatile species that can be correlated with disease progression. If the hypothesis is supported by the experiments, an inexpensive instrument will be developed based on near-infrared (NIR) absorption spectroscopy to provide for long-term, noninvasive continuous monitoring. Successful completion of this project will substantially enhance the diagnosis and treatment regimes for patients developing devastating bacterial infections associated with thermally induced trauma.

(Supported by National Medical Technology Test Bed)

70. Clinical Mutants of Human Sulfite Oxidase

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Sulfite oxidase is critically important for human health. Deficiency results in death, usually at the fetal stage or early in childhood. Individuals that survive into childhood usually have severe neurological abnormalities together with other symptoms such as corneal detachment. We are investigating the structural changes at the active site of sulfite oxidase caused by these clinical mutations with a view to understanding the catalytic mechanism of this important enzyme.

(Supported by NIH)

71. XAS of Copper Chaparones—Menkes Protein and Related Copper Transport Diseases

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Deficiency in copper transport proteins is involved in several, often fatal, diseases, such as Wilson's and Menkes'. We are using XAS to study the structure and mechanism of copper binding to several human copper transport proteins.

(Supported by NIH and Australian Research Council)

Imaging

This large section lists research projects dealing with all types of imaging on both the macroscopic and microscopic levels. Numerous projects address the development of new or improved imaging instruments employing the techniques of positron emission tomography, single photon tomography, standard x-ray and angiographic techniques, ultrasound, magnetic resonance imaging and spectroscopy, optical diffraction and spectroscopy, and x-ray and vibrational spectroscopy. Considerable effort is dedicated to the development of small animal scanners, useful for drug development and potentially suitable for investigations of gene expression. Much effort is also committed to the development of new radioactive tracers for a variety of applications, key among these being cancer detection and neurophysiologic studies. Also included in this section are clinical investigations which use new tracers or new measurement techniques for elucidating physiologic and pathophysiologic processes; a large number of these projects deal with neurophysiology, neurologic disorders, and mental health.

72. The Development of a Compact Source for Dual-Energy Digital Subtraction Angiography

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Diffraction Enhanced Imaging, DEI, is a new imaging modality developed at the National Synchrotron Light Source (NSLS). DEI uses an analyzer crystal following the sample to greatly decrease the effects of scattered radiation on image quality. At the same time, it makes the imaging sensitive to the small angle scattering and refraction properties. The result has been images of mammography phantoms and excised human breast tissues with tumors which have dramatically improved contrast over standard imaging techniques. If this technology could be applied *in vivo* as a screening technology for breast cancer, it might lead to earlier and more positive identification of the disease.

(Supported by DOD ARPA)

73. X-Ray Schlieren Computed Tomography

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A new method of mammography was invented at the National Synchrotron Light Source (NSLS) in 1986 by Chapman, Thomlinson, and their collaborators: Diffraction Enhanced Imaging (DEI). It uses monochromatic, fan-shaped-beam x rays for line-by-line imaging of the subject. At the heart of the method is an analyzer crystal, of the same type as used in the system's monochromator, that is positioned between the subject and the detector. The analyzer allows the detection of only those x rays that did not deviate from their original fan plane beyond the acceptance angle of the analyzer as they passed through the subject's body. This angular-deviation information generates two new images of the subject: a) that of the x-ray index of refraction and b) that of absorption in which the so-called "x-ray small-angle scattering" is suppressed. The first image is a natural "edge-enhancer" that accentuates the boundaries between different types of tissue, while, in the second image, contrast between different tissue types is typically enhanced remarkably because of differences in the amount of small-angle scattering in different tissue types. We implemented this method in the computed tomography (CT) mode at the X15A beamline of the NSLS, imaging phantoms and animal tissues. The goal has been quantitative tissue characterization for future clinical use of DEI in

mammography; the work could not have been done in the planar imaging mode because of the problem of overlying tissues in the planar images.

(Supported by LDRD)

74. High Beam Current, Low Energy Targetry for Production of Radioisotopes for Positron Emission Tomography

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The major focus of this project is the design of targetry modules which will produce acceptable quantities of a given positron-emitting radioisotope using a windowless or thin window design. These targets will be used on a low energy (7 MeV), high beam current (>100 microamp) accelerator being marketed by AccSys Technology, Inc. These targets will enable this accelerator to produce useable quantities of radioisotopes for Positron Emission Tomography (PET). The project team's extensive experience in designing cyclotron targets provides a springboard to the development of these new, more demanding targets. A 4 MeV prototype accelerator supplied by AccSys is being utilized to carry out the design experiments. The critical tasks that must be accomplished are the design of very thin vacuum isolation windows which will withstand the very high pressures generated inside the targets, and of very efficient heat removal systems for use with these targets. The high power density of these low energy, high beam current accelerators makes heat removal the highest priority. Without these targets, the accelerator cannot be used for production of PET isotopes, which would close a very large portion of the potential market for the manufacturer. The successful completion of this project will make PET technology accessible to virtually every modern medical facility by freeing the technology from dependence upon an operating cyclotron.

(Supported by DOE SS/LTR, Partner—AccSys Technology, Inc.)

75. Non-Diamagnetic Agents for In Vivo ^{23}Na and $^{1}H_2O$ MR

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The work proposed will continue to explore and demonstrate the range of applications of the effects of non-diamagnetic agents on the two strongest tissue NMR signals: those of $^{1}H_2O$ and ^{23}Na-aq. In this context, the adjective non-diamagnetic indicates any magnetic behavior (ex. Paramagnetic, superparamagnetic, ferromagnetic, etc.) exhibited in addition to simple diamagnetism. Specific projects in the first general category ($^{1}H_2O$) are proposed. The genesis of some of these ideas derived from early work with ^{23}Na-aq. However, now priority must be given to the former, over analogous studies of the latter. It will be demonstrated that one can make $^{1}H_2O$ MR images of contrast reagent (CR) distribution volumes directly, as well as cytolemmal water permeability coefficient maps, cytoplasmic volume images, and extracellular volume maps. The latter two represent the editing of quantitative (spin density) $^{1}H_2O$ images of the fundamental compartmentalization of biology, which can be radically altered in pathologies such as edemas and tumors. The advance that makes these developments possible is the new technique of combined relaxography and imaging (CRI) recently introduced by this laboratory. The CRI technique is explained in this proposal and is totally general and applicable to longitudinal, transverse, or rotating-frame NMR relaxation. It can be used to study any CR, whether employing the hyperfine or the bulk magnetic susceptibility mechanisms (or both). Longitudinal relaxation and hyperfine CRs are emphasized in this proposal. This program has recently moved to a new institution and a significantly higher level of effort. Thus, in addition to rat experiments, studies of canine (beagle) and primate (baboon) models are proposed. These involve the programmed, stepped IV infusions of CRs approved for humans. The ability to study the larger animals provides the opportunity to address important issues of the scaling of the pharmacokinetics and attainable image resolution. The types of images itemized above will be examined for any tissue present in any selected field-of-view, including various muscle

groups, the liver, a tumor model implanted in rat thigh muscle, the brain, and a tumor model implanted in the rat brain. The latter two require direct intracerebroventricular injection of the CR since it does not cross the blood–brain barrier in normal tissue, and this approach will be applied only to rats and dogs. The tumor models allow assessment of much greater tissue heterogeneity and the fundamental bases of their study by the increasingly popular dynamic CR-enhanced method, which involves bolus IV injections. This work involves aspects of physics, physical chemistry, biophysics, and physiology and has ramifications in the study of a number of pathological conditions, including neurological and cardiovascular disorders.

(Supported by DHHS NIGM)

76. Dopaminergic Brain Function in Alcoholics

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The proposed study will enable us to determine if the DA system is abnormal in alcoholics and the functional consequences of these abnormalities as well as the effects of detoxification. In this proposal we will use PET to evaluate the DA system in alcoholics at rest and during pharmacological activation. We hypothesize that at rest DA abnormalities in alcoholics will not be observed in the DA cells but on the postsynaptic elements and on projection areas and that during DA stimulation responses will be accentuated. To evaluate the DA system at rest we will use a multiple tracer approach to assess in the propagation of the DA signal in a single subject. DA terminals are evaluated using [^{11}C]raclopride, a DA D2 receptor ligand and the activity of regions connected with the DA system are evaulated using 2-deoxy 2-[^{18}F]fluoro-D-glucose ([^{18}F] FDG) during early and late alcohol withdrawal. To evaluate the DA system during pharmacological activation we will measure the response to methylphenidate (MP), a drug that increases synaptic DA by inhibiting DA transporters. Measuring the effects of MP on [^{11}C]raclopride binding and on brain glucose metabolism enables us to assess relative changes in DA induced by MP and the metabolic measure will allow us to assess the response of the brain to changes in DA concentration. This knowledge will provide an essential context for understanding the neurochemical mechanisms underlying alcoholism.

(Supported by NIAAA)

77. Positron Emission Tomography (PET) in Cocaine Abuse

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Cocaine is considered as one of the most reinforcing and addictive drugs of abuse. The reinforcing properties of cocaine appear to be associated with its inhibition of the dopamine transporter (DAT). Methylphenidate (NIP), a drug given to children for the treatment of attention deficit disorder, has similar properties at the DAT as those of cocaine, and concerns have been raised about its abuse liability. Though the abuse of NIP has been reported, it is rare, and it predominantly occurs via intravenous administration. We postulate that while inhibition of the DAT by cocaine and MP is indispensable for their reinforcing effects, it is their pharmacokinetics that modulate their addictive potential. More specifically, we suggest that while the rate for peak uptake in brain will influence the intensity of the initial response, the rate of clearance will affect the intensity to subsequent responses during repeated administration. The purpose of this grant is to compare cocaine and NIP with respect to: their regional brain pharmacokinetics, degree of DAT inhibition and potency to change synaptic DA concentration after single and after repeated administration. We propose to use PET in conjunction with [^{11}C]cocaine and [^{11}C]methylphenidate to measure binding parameters and brain pharmacokinetics, [^{11}C]d-threo-methylphenidate to measure DAT occupancy and [^{11}C]raclopride to measure changes in synaptic dopamine (DA) concentration in response to cocaine and NIP. Our working hypotheses are as follows: 1)[^{11}C]cocaine and [^{11}C]methylphenidate will be of similar potency in inhibiting binding to the DAT. (2)Peak uptake and time to reach peak uptake in brain will be similar for [^{11}C]MP and for [^{11}C]cocaine, but clearance of [^{11}C]MP will be significantly slower than that of [^{11}C]cocaine. Slower clearance will lead to a

longer period of DAT inhibition for NIP than for cocaine. (3 After single administration, both drugs will induce similar increases in synaptic DA(as measured by inhibition of [^{11}C]raclopride binding) whereas with sequential drug administrations (at 20 and 60 minutes intervals), NIP will induce a smaller response than cocaine. Preliminary work from our laboratory supports these working hypotheses. Despite the widespread use of NIP in the treatment of attention deficit disorder, very little is known about its pharmacokinetics in brain as well as its abuse liability. In addition to being valuable in understanding the pharmacokinetics and the potential addictive properties of NIP, these studies are relevant in understanding the role of pharmacokinetics of drugs of abuse and therapeutic drugs in their ability to induce repeated self administration.

(Supported by NIH/NIDA)

78. PET Studies of Brain Dopamine in Cocaine Abusers

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While dopamine (DA) appears to be crucial for cocaine reinforcement, its involvement in the loss of control and compulsive administration of cocaine in the cocaine addict is much less clear. Using PET we have shown persistent reductions in striatal DA D2 receptors, which are predominantly located on GABA cells, in cocaine abusers. This finding coupled to GABA's role as an effector for DA led us to postulate that the GABA system participates in cocaine addiction. We hypothesize that chronic cocaine administration leads to disregulation of GABA function, which in turn affects GABA modulation of DA as well as cerebral response to DA stimulation. In this grant we propose to evaluate the GABAergic system in cocaine abusers during withdrawal and detoxification and the effects of treatment with a GABA enhancing drug. We will assess the GABA system indirectly by monitoring the regional brain metabolic response (measured with 2-deoxy-2[^{18}F]fluoro-D-glucose and PET) to lorazepam, a benzodiazepine drug which enhances GABA activity. We will compare the effects of lorazepam to those of placebo in cocaine abusers (n=30) tested 1-2 weeks and then retested 6-8 weeks after last cocaine use. Normal controls (n=20) will be tested in parallel (years 1-3). In years 4-5 we will evaluate the effects of a 6-week treatment with gamma-vinyl GABA (GVG), a GABAergic enhancing drug, on the brain's response to lorazepam in cocaine abusers (n=30). Our working hypotheses are: (1) Cocaine abusers will have an increased sensitivity to GABAergic stimulation secondary to adaptations from decreased GABA function. This will appear as an enhanced brain metabolic response to lorazepam that will be regionally specific and will remain after detoxification. (2) Treatment with GVG will serve to normalize the GABAergic disregulation and restore the secondary adaptation processes. Hence GVG treatment will decrease the enhanced regional metabolic sensitivity to lorazepam in cocaine abusers. Studies in laboratory animals and pilot work from our laboratory in cocaine abusers support these hypotheses. Furthermore, because in laboratory animals GABA enhancing drugs decrease the reinforcing effects of cocaine and attenuate cocaine-induced increases in DA, findings from these studies are of relevance not only from the perspective of understanding neurochemical substrates of addiction but also for the development of new treatments for cocaine abuse.

(Supported by NIDA)

79. Pharmacokinetics of Psychostimulants & Reinforcement

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Cocaine and methylphenidate (MP) are both psychostimulant drugs. Yet cocaine is considered one of the most reinforcing and addictive drugs of abuse, while MP is widely used to treat attention deficit disorder in children. MP has a similar affinity for the dopamine transporter (DAT) as cocaine. Preliminary work from our laboratory using PET and [^{11}C]cocaine and [^{11}C]methylphenidate showed a striking similarity for the pattern of distribution of these two drugs in the human brain with competition for the same binding sites. However, they differ in their pharmacokinetics. MP's clearance from brain is significantly slower than that of cocaine. We hypothesize that the slow clearance of MP in brain will interfere with the

magnitude of the response to a subsequent dose of MP, or cocaine, thus decreasing the likelihood of "bingeing" behavior. We propose to test these hypotheses by investigating the relation between the pharmacokinetics of MP in brain, its inhibition of the DATs and its behavioral effects after single and repeated administration. We will also investigate the effects of MP administration on the binding of cocaine in brain. We propose to use PET in conjunction with: [^{11}C]methylphenidate to measure brain pharmacokinetics of MP, d-threo-[^{11}C]methylphenidate to measure DAT occupancy after single and repeated iv MP administration and [^{11}C]cocaine to evaluate binding inhibition by pharmacological doses of MP. For this last experiment we have chosen to use oral administration rather than iv because of the potential usefulness of a "slow release" form of MP in the treatment of cocaine addict. In parallel we will also evaluate the effects of oral MP on the behavioral response to a pharmacological dose of iv MP. Our working hypotheses are as follows: (1) The initial uptake of MP, as well as the fast inhibition of the DAT by MP, will be associated with the "high," but the continuous inhibition of the DAT will not. When a second dose is administered while the DATs are still inhibited, the "high" will be significantly decreased despite equivalent or even a larger percent of DAT inhibition. (2) Oral MP administration will inhibit binding of [^{11}C]cocaine and [^{11}C]MP to a comparable extent and will decrease the magnitude of the iv MP-induced "high." Understanding the relation between MP's pharmacokinetics in brain, its length of occupation of the DAT and its psychoactive effects is important not only in understanding the addictive potential of MP and other psychostimulant drugs but also for the development of therapeutic strategies to treat cocaine addiction.

(Supported by NIDA)

80. Physiological Imaging

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This project will take advantage of the imaging technologies available at BNL to investigate physiological and neurochemical mechanisms underlying human brain function as well as the effects of psychoactive drugs on these processes. Our investigations focus on the role of dopamine (DA) in substance abuse and in aging, and, more recently, obesity. The specific aims of this work include: 1. Characterization of the molecular changes underlying addiction (and obesity) and their relationship to brain function and treatment of addictive (and eating disorder) behavior. 2. Gain understanding of the molecular changes underlying normal aging to relate those processes to vulnerability to neurodegenerative disease and treatment. 3. Facilitate new drug development by gaining a clear understanding of drug pharmacokinetics and pharmacodynamics. 4. Integration of different imaging modalities to the investigation of functional brain abnormalities associated with aging and addiction.

(Supported by DOE OBER)

81. PET Investigations of Neuro Transmitter Interactions

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This project is designed to use positron emission tomography (PET) to investigate the functional linkage of neurotransmitter systems. Specifically, it will focus on the interactions of dopamine and serotonin since both systems have been implicated in schizophrenia, depression and affective disorders. The applicants will use PET to quantify the responsiveness of dopaminergic and serotonergic systems to specific pharmacologic challenges. These studies will use [^{11}C]raclopride (RAC), a D2/D3 receptor antagonist, to image postsynaptic dopamine receptors and a new ligand, [^{11}C] SR46349B, a serotonin (5-HT2) antagonist. By observing dose response and time course of events, the ability of PET to quantify pharmacologic activity will be validated by assessing the following: (1) the sensitivity of the response at different challenge doses, (2) the reproducibility of response, (3) the stability of the baseline measurement, and (4) the time course of response to the challenge. Also, the effect of anesthesia on these interactions will be investigated. Finally, findings in non-human primates will be confirmed by using in vivo microdialysis in freely moving rats to study changes in endogenous neurotransmitter levels.

(Supported by NIH)

82. PET in Schizophrenia

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Positron emission tomography (PET) will be used to investigate the capacity to alter neurochemical function in response to acute neuroleptic-induced receptor blockade in chronic schizophrenia. A neuroleptic challenge paradigm will be used to test the hypothesis that the capacity to alter neurochemical function in response to acute, neuroleptic-induced receptor blockade is associated with the capacity to produce a therapeutic response to long-term neuroleptic treatment. PET and ^{18}F-fluoro-2-deoxyglucose (FDG) will be used in a repeated measures design to examine regional changes in glucose metabolism following administration of a single pharmacological challenge dose of the neuroleptic drug haloperidol sufficient to induce receptor blockade. By comparing the metabolic response to this challenge in clinically well-characterized treatment responsive and treatment resistant chronic schizophrenics, we will address our central hypothesis. PET and the muscarinic cholinergic ligand ^{11}C-benztropine (^{11}C-BZ) will also be used to measure regional changes in muscarinic activity following administration of the haloperidol challenge in order to characterize the effect of a dopamine-blocking neuroleptic challenge on the separate, but functionally-related muscarinic cholinergic system. By comparing the muscarinic response in treatment responsive and resistant schizophrenics, one aspect of our central hypothesis can be tested: that the capacity to alter neurochemical response to receptor blockade in a separate but functionally related neurotransmitter system is associated with the capacity to produce a therapeutic response to long-term neuroleptic treatment. By accomplishing these aims we will have defined a neurochemical measure that may predict drug treatment response in chronic schizophrenia. Such a measure may provide a neurochemical basis for interpreting treatment response, for clinical sub-typing and for developing new treatment strategies.

[Supported by New York University (NIH)]

83. PET Studies of Catechol-O-Methyltransferase

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This proposal involves the development of radiolabeled catechol-o-methyltransferase (COMT) inhibitors for positron emission tomography (PET) studies of the enzyme system in vivo. COMT regulates the concentration of neurotransmitters in the central and peripheral nervous systems and is an important molecular target in the development of drugs to treat Parkinson's disease (PD) since its metabolism of L-DOPA limits drug bioavailability. COMT is distributed throughout the body and brain, and abnormalities in its activity may be associated with neurological, psychiatric and cardiovascular disorders. It is also elevated in breast cancer tissue where it plays a role in estrogen metabolism. There is limited information on the regional distribution of COMT or of changes in its activity occurring in diseases. The Brookhaven PET Group has recently developed a synthetic route to ^{18}F-labeled Ro41-0960, a potent and selective COMT inhibitor. Initial PET studies demonstrated its ability to label the COMT sites in organs such as kidney, liver and heart. The goals of this proposal are to further characterize ^{18}F-Ro41-0960 as a tracer for peripheral COMT in vivo; synthesize and characterize ^{11}C-labeled Ro40-7592, a COMT inhibitor used clinically to treat PD; and to examine the pharmacodynamics of COMT inhibition wih PET. Proposed studies include the measurement of baseline COMT distribution and the assessment of the sensitivity of the radiotracers to changes in COMT activity. The hypothesis is that labeled COMT inhibitors will map COMT in vivo, and the hypothesis will be tested by a complementary ex vivo approach in which radiotracer uptake will be correlated with COMT activities in rodents. The proposed studies will provide the first functional maps of COMT in the primate, thus affording a scientific tool for the investigation of COMT in living systems along with potential applications to drug development and oncology.

(Supported by NIH/NINDS)

84. PET Imaging of Estrogen Metabolism in Breast Cancer

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Malignant cells grow out of control because the normal biochemical processes regulating growth are disrupted. A knowledge of the disrupted biochemical processes is key to understanding how cancer develops, to detecting it at an early stage and to developing and monitoring treatment. One of these processes is estrogen metabolism. It is well known that many breast tumors depend on estrogen, and there is now evidence that the presence of estrogen metabolites (catecholestrogens) in breast tumors cause changes in the DNA that may lead to uncontrolled cell growth. Catecholestrogens are broken down by catechol-o-methyltransferase (COMT). COMT is elevated in malignant breast tumors, and abnormal COMT genes have been found in individuals with breast cancer. We developed ^{18}F-labeled Ro41-0960 as a radiotracer for visualizing COMT with positron emission tomography (PET). Here we propose to investigate whether PET-visualized COMT activity can serve as a molecular marker for breast cancer and thus aid in the diagnosis of breast cancer. To accomplish this we have two specific aims: (1) to perform studies in breast tumor tissue samples to determine whether the binding of [^{18}F]Ro41-0960 correlates with the activity of COMT and (2) to determine feasibility of PET imaging COMT in human breast cancer patients with grade III breast carcinomas. We predict that the uptake of [^{18}F]Ro41-0960 by breast tumor tissue will be substantially greater than that by normal surrounding tissue and that in breast cancer patients tumors will be visualized with PET owing to the significantly greater tracer uptake in tumor relative to surrounding normal tissue. This novel approach goes beyond diagnosis to delineate the fundamental biochemical properties and molecular signatures of tumor cells. If successful this new method can reduce the need for unnecessary breast biopsies and enhance the staging capabilities of current diagnostic procedures. Additionally, the discovery of the biochemical processes involved in the deregulation of cell growth may suggest new treatment opportunities.

(Supported by DOD)

85. Neuroreceptor Radioligands and Synaptic Activity

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Our overall goal is to develop methodology for examining synaptic biology, specifically the concentration of neurotransmitters and their receptors, using Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT). This will lend depth to the results of nuclear imaging studies by providing a perspective on the molecular mechanisms underlying normal and abnormal synaptic biology. Changes in both neurotransmitter and receptor levels alter the uptake of receptor radioligands. Although techniques such as microdialysis and electrophysiology can be used to assess synaptic activity in animal models, these are not applicable to individual human patients. The importance of non-invasive *in vivo* radioligand binding technologies such as PET and SPECT is that they can be applied to humans and therefore provide a bridge between experimental and pre-clinical studies and medical practice. The feasibility of monitoring neurotransmitter release will further help in the evaluation of neuropsychiatric disorders and of pharmacological treatment.

(Supported by DOE OBER)

86. Estimation of Synaptic Dopamine Using PET & SPECT

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Binding of dopamine D2 PET and SPECT radioligands including [^{11}C]raclopride and [^{123}I]IBZM in vivo is affected by competition with endogenous dopamine. This allows changes in synaptic cleft levels of dopamine to be detected in human subjects, via corresponding increases or decreases in radioligand binding. If the factors which affect competition

between exogenous radioligands and neurotransmitters were better understood, quantitative measures of the average concentrations of dopamine in the synaptic cleft could be obtained in conditions such as substance abuse and schizophrenia. As yet, however, the information needed to relate changes in D2 radioligand binding to changes in dopamine is lacking. Based on preliminary studies, it is proposed to further develop a superfused brain slice preparation, in which synaptic cleft neurotransmitter levels can be manipulated by controlling the rate of external electrical stimulation to the slice, while radioligands and pharmacological agents can be administered under very controlled conditions, including maintenance of radioligand concentrations at defined and constant values. The slices contain living cells with apparently normal synaptic connections, so that the local factors affecting competition in vivo are believed to be retained. The preparation represents a model of tomographically isolated brain tissue. The proposed experiment will thoroughly characterize the binding of several D2 radioligands to the slices, in terms of the kinetics of association and dissociation, and equilibrium binding parameters. Effects of electrical stimulation of the slices, of the dopamine reuptake blocker cocaine, and of the dopamine releasing drug amphetamine on radioligand binding will be evaluated. The influences of experimental reduction in D2 receptor density, of radiotracer affinity and of equilibrium versus non-equilibrium radioligand binding will also be examined. In addition to radioligand binding, a functional assay of dopamine concentration, the inhibition of electrically evoked acetylcholine release, will be employed. The average dopamine concentrations corresponding to a range of stimulation frequencies will be assessed using equilibrium radioligand binding models, and some of the anticipated conclusions of the studies will be tested in an animal model. The proposed studies are important both for the design and interpretation of PET/SPECT studies with currently available radioligands, and for elucidation of the factors necessary for design of optimum radiotracers for neurotransmitter competition experiments.

(Supported by NIH)

87. Modulation of Neurotransmitter Release by Cannabinoids

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Marijuana is one of the most commonly used drugs of abuse in the United States. The active ingredient of marijuana, delta 9THC, acts on specific receptors, termed cannabinoid receptors, which are abundantly expressed in many regions of the brain. Although relatively few studies have been performed to determine their function in these regions, a principle role for these receptors appears to be in the presynaptic regulation of neurotransmitter release. Our own preliminary data examining the effects of cannabinoids on neurotransmitter release from rat brain slices suggests that cannabinoid receptor activation produces a marked inhibition of the release of ACh in the hippocampus and also to a lesser extent in the nucleus accumbens. The strong inhibitory effects of cannabinoid receptor activation on hippocampal ACh release may account for the well-known short-term memory impairment resulting from marijuana use. In addition to the extensively documented behavioral effects of cannabinoid agonists, recent behavioral and electrophysiological observations in rodents have indicated that a newly developed cannabinoid antagonist, SR 141716A, produces effects on its own in the brain. This suggests the presence in the brain of an endogenous tonic cannabinoid. In our studies we have found that SR 141716A and the related compound, AM281, on their own produce a potent and large enhancement of ACh release in hippocampal slices. Taken together these observations thus suggest that this new class of compounds may have therapeutic uses distinct from those of cannabinoid agonists (for example, ameliorating the memory deficits seen in Alzheimer's disease). In this proposal we will extend our preliminary findings on the effects of cannabinoid receptor ligands on neurotransmitter release. Our specific goals are two-fold. First, we will use ACh release from hippocampal slices as a CNS assay system to evaluate the efficacy of new cannabinoid compounds developed by A. Makryannis. Second, we will obtain an explanation for the approximately two-fold enhancement of ACh release from the hippocampus produced

by low concentrations of SR 141716A and AM281. This suggests either that cannabinoid receptors are constitutively active and produce a tonic inhibition of ACh release or else that these antagonists are antagonizing the effects of an endogenous agonist that is co-released by electrical stimulation of the slices. For the latter case an examination of the effects of new anandamide uptake inhibitors and amidase inhibitors in the slice preparation would help to confirm this compound as the endogenous ligand for cannabinoid receptors. These experiments will thus provide data using a biologically relevant in vitro CNS preparation on the potency and efficacy of newly developed cannabinoid receptor ligands and this in turn may provide information that is of value in understanding how marijuana produces its psychotropic effects in the brain. Furthermore, these experiments will give information on the mode of action of cannabinoid antagonists and could suggest novel therapeutic uses for this new class of compounds.

(Supported by NIH)

88. Enhancement of Functional and Neurochemical Brain Pattern

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The need to consolidate and integrate information on the functional, neurochemical and anatomical organization of the brain is widely recognized. The objective of this research is the development and validation of a fast and accurate computer-based method to detect and analyze faint whole brain 3-dimensional signals in PET and MRI images. Such methods will increase our capacity to detect functional and neurochemical patterns associated with activation studies, drug intervention or mental illness. They will also enable the generation of data bases for the study of variability in anatomical structure, metabolic and neurochemical patterns. Our working plan will achieve the following specific aims: a) improve the computing speed over existing methods, while minimizing the need of user supervision; b) improve the enhancement of the signal to noise ratio in group-specific brain patterns over that of available existing methods; c) develop a strategy that avoids the need to select reference landmarks; d) develop a method that will have low sensitivity to camera resolution, (FWHM), image noise, camera slicing angle or missing brain slices; and e) develop an automated method that will accurately project selected functional or neurochemical patterns into a brain anatomical atlas (cadaver or MRI based). Our preliminary studies support the feasibility of achieving these aims. The basic strategy is to develop methods that reduce the intersubject variability due to patient positioning and the brain's functional, neurochemical and structural variability across the group. Taking advantage of the large variety of different radiotracers at Brookhaven National Laboratory, these methods will provide a unique perspective to individual data which has been collected over the past ten years. To begin we will examine group patterns for the following tracers: FDG, ^{18}F-N-methylspiroperidol, ^{18}F-NMS, ^{11}C-cocaine, ^{11}C-raclopride, ^{11}C-L-deprenyl and ^{11}C-benztropine. The feasibility of obtaining group, or disease specific, neurochemical and/or functional brain maps will represent a unique resource to the neuroscience and neuropsychiatric community.

(Supported by NIH)

89. Radiotracer Research and Development in Nuclear Medicine and Neurosciences

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The Brookhaven PET program has its roots in the Chemistry Department and in basic research in chemistry and is a core element of the Brookhaven Center for Imaging and Neuroscience. A unifying central theme is the development of positron emitting labeled radiotracers as scientific tools for mapping biochemical transformations and the movement of drugs in living systems. Basic elements of the programs include: *Radioisotope Research*: To advance the production of medical radioisotopes; to explore cryogenic targets for production of short-lived positron emitters (C-11, F-18, N-13); to develop targetry for low energy, high beam current accelerators; *Radiotracer Synthesis with Short-Lived Isotopes*: To develop new methods for increasing reaction rates, introducing energy, reducing reaction

scale, influencing stereochemistry, facilitating purification, and predicting reactivity; *Basic Neuroscience*: To advance the understanding and prediction of the interactions between chemical compounds and biological systems; to develop *in vivo* methods for probing the neurotransmitter interactions; *Radiotracer Design and Neuroimaging*: To design radiotracers with optimal properties for quantitatively imaging different biological targets; to understand and model radiotracer kinetics and their relationship to biochemical transformations; to characterize the molecular changes underlying drug action, drug addiction, normal aging and neurodegeneration; to advance the develoment of therapeutic drugs and; *Synergistic Imaging*: To integrate PET and MRI to combine the unique features of both.

(Supported by NIH)

90. ^{1}H Spectroscopic Imaging of Multiple Sclerosis

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Multiple sclerosis (MS) is a disease of the central nervous system white matter in which demyelination is the major pathologic process. This demyelination results in delayed and/or blocked signal transmission, causing the clinical findings such as blindness, weakness and numbness. It has presumably an immune etiology, although the specific processes have yet to be determined. Two aspects of this disease will be evaluated in this study. First, although objective criteria for distinction remain unclear, clinically, several subtypes of disease exist in MS. It is hypothesized that these subtypes may be demonstrable by magnetic resonance spectroscopy (MRS). Second, the recent development of multiple new therapies for the treatment of MS makes it critical to have objective measures of fluctuating disease.

(Supported by Multiple Sclerosis Society)

91. Cerebral Metabolism in Ketosis and Epilepsy: ^{1}H and ^{31}P Spectroscopy Study at 4.1T

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Epilepsy has a prevalence of 5 to 8 per 1000 population. In pediatric patients, uncontrolled seizures are associated with brain damage, mental retardation, and sudden death. Most therapeutic approaches are based on medication or surgery. However, in intractable epilepsy, medications are often inadequate or plagued with side effects and surgery is not an option, based on seizure type and cause. The ability of the ketogenic diet (high fat) to treat the most intractable epilepsies has made it a source of hope for families, such that it has been a topic of movies and documentaries. It is actually a very old method, dating to biblical times, although the study of how the diet causes its effect has only been a recent undertaking. The diet does not cause weight gain; its major drawbacks are an increase in serum lipids and cholesterol and its palatability. However, its clinical efficacy has been well documented, although its mechanism remains poorly understood. The proposed Magnetic Resonance Spectroscopy (MRS) study will examine various hypotheses: (1) that the diet results in increased cerebral energy; (2) that the diet changes cerebral amino acid levels. Thus, this project will evaluate the origin of improved seizure control with a ketogenic diet by assessment of cerebral energy levels (^{31}P MRS) and amino acids (^{1}H MRS).

(Supported by Charles A. Dana Foundation)

92. Passive Shim Arrays for Improved Static Field Homogeneity in Magnetic Resonance Imaging of the Human Head

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The purpose of this research is to determine a distribution of high-susceptibility compartments, arrayed proximal to the human head, that will improve the attainable static magnetic field homogeneity in the human brain for Magnetic Resonance Imaging (MRI) applications. The objective for the High-Field MRI Laboratory at BNL is to develop improved methods for quantitative MRI with full brain coverage. These improvements will allow mapping of brain structure and function with much greater accuracy than is presently possible. The objective for Advance Imaging Research, Inc. is to modify the design of a radio-frequency coil so that the array of high-susceptibility compartments does not produce a net perturbation to the coil's radio-frequency field.

(Supported by DOE SS/LTR; Partner—Advance Imaging Research, Inc.)

93. Applications of Quantitative MRI: Water Content and Blood Brain Barrier Permeability in Multiple Sclerosis

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Multiple sclerosis (MS) is a demyelinating disease of the central nervous system that directly affects an estimated 350,000 people in the United States. MRI is the imaging modality of choice in the diagnosis of MS and detects focal white matter lesions with unsurpassed sensitivity. This sensitivity has been exploited to test promising new therapies for MS, and MRI is now routinely used as a primary outcome measure in clinical trials. For these applications the use of MRI has been qualitative (are there any new white matter lesions?), or at best semi-quantitative (how many new white matter lesions?). Recent quantitative MRI evidence suggests that MS white matter devoid of lesions (so-called normal appearing white matter, NAWM) is also abnormal. Very recent evidence suggests that NAWM areas reporting the *most* abnormal MRI measures go on to develop new focal lesions within a few months. Though never directly reported, most of the quantitative MRI evidence (increased water proton relaxation times, increased apparent diffusion coefficients, etc.) is consistent with the view that parenchymal water content is increased in MS NAWM. It has long been appreciated that focal disruption of the normally tight blood–brain barrier (BBB) is a pathological hallmark of MS. The importance of this recent evidence is that it suggests that a diffuse, though subtle, increase in BBB permeability *precedes* macroscopic disease progression. There are two possible interpretations of this abnormality 1) it is an inherent difference that is causative of the disease (i.e. increased BBB permeability renders MS brain more susceptible to demyelination), or 2) it represents microscopic disease that is secondary to the primary disease process. This application proposes clinical studies of MS patients and healthy controls to determine: a) if brain water content is increased in MS, b) if blood–brain barrier (BBB) permeability is increased in MS brain, and c) if increased water content and BBB permeability are predictive of disease progression. We will also test whether these changes represent a primary underlying (perhaps causative or predisposing) condition or a secondary response to active disease. The broader objective of this research is to continue to develop and refine quantitative magnetic resonance imaging techniques; this area has been one of the fundamental strengths of BNL's High-Field MRI Laboratory. As such, the project summarized above represents an important application of these quantitative methods. Expected outcomes of this research include increased understanding of the fundamental pathology of MS, improved outcome measures to track therapeutic interventions in MS, and improved methods to extract quantitative information from MRI.

(Supported by LDRD)

94. High-Field Magnetic Resonance Imaging

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The enormous impact of imaging on basic research in the neurosciences and in the diagnosis and treatment of brain disease led to the decision to establish a state-of-the-art Imaging Center at BNL. The Brookhaven Center for Imaging and Neuroscience is dedicated to basic and biomedical research and to integrating data from positron emission tomography (PET), magnetic resonance imaging (MRI), and single-photon emission computed tomography (SPECT), among others, in order to investigate the synergistic uses of multiple imaging modalities in studies of the functioning brain of humans and animals, as well as the functioning of other organs. The Imaging Center has been built upon the Brookhaven PET program, expanding it to include MRI and SPECT. The fully integrated Center is being developed in stages. A recent stage is the establishment of a High-Field MRI Laboratory. The building to house the Laboratory was completed in FY 96. An MRI instrument that utilizes a superconductor magnet with a field strength of 4 Tesla, the largest used for humans, has been installed and commissioned in stages. There are only seven other 4 T machines in the world. With only one remaining capital supplement, the BNL scanner will be a cutting-edge instrument for activation studies, for *in vivo* spectroscopy and for further developing relaxographic imaging, which was originated in this MRI group. The Brookhaven Center for Imaging and Neuroscience is currently developing new forms of imaging and experimental strategies for investigating *in vivo* molecular mechanisms that go beyond the confines of a single imaging method. As just one example, combining the unique ability of relaxographic MR imaging to map water volumes in tissue with the unique ability of PET to detect minuscule amounts of a rich variety of labeled molecules is a major effort and will ultimately allow for the first time the determination of true concentrations *in vivo* without the requirement for physical sampling. Other mechanistic studies are directed toward mapping the effects of neuro active drugs including anesthetics and substances of abuse, on the functional activity of the brain.

(Supported by DOE OBER)

95. ^{1}H NMR Chemical Shift Imaging in Temporal Lobe Epilepsy

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Epilepsy is one of the most common neurological disorders affecting between one and two million Americans. For approximately ten percent of these patients, in which medication fails to control their seizures, surgery often proves to be an effective treatment. However, the number of patients receiving such treatment is limited by the difficulty and expense of the diagnostic procedures available for localizing the seizure focus. Recent ^{1}H NMR spectroscopy studies have demonstrated that measurements of metabolite ratios N-acetyl aspartate (NAA)/creatine plus phosphocreatine (CR) (NAA/CR ratio) and NAA content provide a sensitive marker for lateralizing the seizure focus in patients with intractable temporal lobe epilepsy (TLE). These studies have typically utilized large single volume measurements (eliminating a regional assessment of the extent and severity of the disease) or relied on relative differences between the two hemispheres (compromising the ability to assess cases of bilateral disease). Therefore significant enhancements in the predictive value of NMR spectroscopy as applied to TLE can be expected if absolute measurements using regional mapping methods can be developed and applied. Therefore, the specific aims of this project are to: (1) extend and evaluate statistically based ^{1}H CSI methods to identify the location and extent of the epileptogenic issue using the NAA/CR ratio; (2) quantify metabolite changes in mM/kg in CR and NAA to determine the origin of the ratio changes; (3) investigate the diagnostic utility of glutamine and glutamate measurements in localizing the seizure focus; and (4) determine the sensitivity, specificity and additive value of these methods with respect to scalp EEG and MRI at 1.5T. The findings from these aims will be correlated with histologic evaluation of the resected tissue.

(Supported by NIH)

96. Regional Uptake Kinetics and Visibility of Brain Alcohol

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One of the mechanisms by which ethanol conveys its intoxicating effects is hypothesized to be through its interaction with cerebral membrane lipids. As such, the nature of ethanol's interaction with cerebral membrane lipids, and their effect on the NMR relaxation properties of ethanol, are of considerable interest. A number of investigators have reported that 73-79% of brain alcohol is not visible *in vivo*. It is believed that this "invisibility" is due to an anomalously short T2 arising from the interaction of ethanol with the membranes. Additionally, the fraction of invisible alcohol has been linked with a variety of its pharmacological effects, including long- and short-term tolerance. However, after nearly a decade of investigations the measurements of visible alcohol *in vivo* vary widely, depending upon the pulse sequence and the biological model used. The variability in the reported alcohol levels is most likely due to differences in the pulse sequences used, corrections for relaxation and J-modulation and the internal reference employed for concentration estimates. Depending on the pulse sequence used, type of refocusing pulses used, and echo time, significant losses in signal intensity can occur for J-modulating systems such as ethanol. Therefore, the goal of this study was to evaluate the regional visibility of ethanol in the human brain employing a spectroscopic imaging sequence that minimizes T1, T2 and J-modulation differences in comparison to an internal reference (NAA). After establishing the baseline visibility, the relationship between ethanol visibility and acute and chronic tolerance will be investigated.

(Supported by LDRD)

97. High Intensity LINAC Electron Beam for Generating High Intensity X-Rays for Transvenous Coronary Angiography

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The high intensity electron linac currently operating in the Fermilab Accelerator Test Facility at A0 is well positioned to investigate accelerator improvements that could lead to a hospital-based accelerator for transvenous coronary angiography, which is much safer than arterial coronary angiography. At present large synchrotron radiation sources are being used to investigate the transvenous technique, but synchrotrons are too large to be practical in a hospital. German scientists from Darmstadt who are collaborating on the A0 project have actively investigated the possibility of using electron linac beam coupled with channeling radiation. This project has the potential of making safe angiography more widely available.

(Supported by internal funds)

98. High Resolution Biomedical Imaging System with Direct Detection of X-Rays Via a Charge Coupled Device

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Principal investigator, Muzaffer Atac has patented this device that images biological tissue without the use of x-ray film. An energy source emits x-rays that pass through the tissue and are detected by a charge-coupled device (CCD) located immediately adjacent to the tissue. The device provides better resolution than is available from similar commercial devices.

With more development this device could be used to obtain better resolution in mammography. A related application, which could be developed at The Neutron Therapy Facility (NTF), is the use of CCD's to provide verification images of patients being positioned for radiation therapy. This would eliminate the need for films, which ultimately must be recycled, and it would speed up the process of treating patients. We are discussing the possibility of developing the technology to generate CCD images rather than using port films at NTF.

(Supported by internal funds)

99. PPG Project I: Myocardial Viability and Perfusion Reserve Imaging

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The purpose of this project is to develop and apply improved methods of myocardial perfusion quantitation, myocardial flow reserve and wall dynamics for the evaluation of the effects of coronary artery disease. The protocols emphasize flow quantitation using PET with applications to detection of dysfunctional reversibility and to quantitation of coronary perfusion reserve. A major hypothesis is that quantitative PET perfusion is able to show the likelihood that dysfunctional myocardial segments will recover following revascularization procedures and that perfusion quantitation is superior to fluorodeoxyglucose accumulation patterns or other contemporary non-invasive approaches being used to assess viability. Forty patients who are to receive by-pass surgery will be studied. A new perfusion tracer using the $^{122}Xe/^{122}I$ generator could replace the need for an on site cyclotron and make PET perfusion studies markedly more economical and practical in view of the new sources for ^{122}Xe. The technological improvements in MRI could challenge PET methods for evaluation of ischemic heart disease and perfusion reserve thus these MRI methods will be compared to the best of the PET methods in years 4 and 5. Four cardiologists (three with the nuclear medicine specialty) and a cardiac radiologist have been included in addition to the medical scientists developing needed technology.

(Supported by NIH/NHLBI, P01 grant)

100. Experimental Medicine Clinical Diagnostic

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Advanced nuclear medicine and NMR technologies for investigating mechanisms of aging, atherosclerosis, heart disease, mental disorders and cancer. This FTP focuses on design and data analysis for advanced positron (PET) and single photon emission tomography and magnetic resonance imaging systems which have capabilities beyond those currently envisioned for commercial implementation. The joint approach of imaging methods and isotope technologies is applied to medical science problems using a team of physicists and research physicians devoted to development of quantitative methods of experimental medical science. Results of this FTP include the development of the Anger camera, dynamic PET, quantitative reconstruction algorithms, compartmental modeling methods, a unique 2.6 mm resolution PET instrument, PET isotope generators, and determination of safety criteria for human exposures to very high static and oscillating magnetic fields. Medical applications include whole body PET evaluation of patients with cancer, heart disease, Alzheimer's disease and schizophrenia. Three instrument development projects are supported by this FTP: PET breast cancer imager, 2 mm resolution PET and a proposed 10T whole body magnet. Proposed work includes deployment of the Xe-122/I-122 PET generator for practical brain and heart blood flow imaging, PET aptamer biodistribution studies, imaging studies of gene expression, and boron compound biodistribution studies for neutron capture therapy (BNCT). This FTP addresses problems where advances in computational science are needed to aid

biological and medical research with complex instrumentation systems.

(Supported by DOE, Field Task Proposal)

101. I-122 Generator and Radiotracers for Perfusion Studies

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Heart and brain perfusion imaging before and after stimulation (e.g., dipyridamole and acetazolamide, respectively) is a clinically important procedure for the efficient care of patients with vascular diseases. Whereas over 15 different methods have been employed ranging from x-ray computed tomography with inhaled stable xenon to transcranial Doppler ultrasound, none has the attributes of resolution, quantitation, volume of organ coverage and widespread practical availability. This 5-year project is to develop the iodine-122 (3.6 min) positron emission tomography (PET) tracers particularly for heart perfusion by perfecting the rapid iodination chemistry and by technological improvements of the ^{122}Xe/^{122}I radioisotope generator such that ^{122}I-compounds can be reliably and automatically delivered. ^{122}Xe (20 hr) availability from the University of California at Davis and potential availability from other accelerators has motivated this research project. Brain perfusion studies with ^{122}I-HIPDM have been completed in 16 normal subjects, 5 Alzheimer's patients and 1 stroke patient. Cardiac perfusion agents will be selected from the best of ^{122}I-labeled amphetaminium, piperazinium and imipraminium quaternary amines and rotenone classes of compounds after studies in red cell perfused rabbit heart preparations and dog PET studies. Comparisons of the selected compound will be made against $^{13}NH_3$ and ^{62}Cu-PTSM by dynamic PET in man.

(Supported by NIH/NHLBI, R01 grant)

102. Compact Gamma Camera System for Breast Cancer Imaging

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This instrumentation development proposal is relevant to both the detection of breast and axillary node cancer and the identification of tumor regression or recurrence after surgery, irradiation, or chemotherapy. The superior image resolution and ability to take a greater variety of oblique views will allow the detection of both smaller tumors and smaller changes in existing tumors. The low cost of the proposed camera, coupled with the availability of ^{99}Mo-^{99m}Tc generators and kits for preparing tumor imaging agents such as ^{99m}Tc-sestamibi, should make this clinical resource widely available. The innovative aspects of the proposed instrumentation include the following: (1) Novel low-noise silicon photodiode arrays (developed at LBNL) that are illuminated from the unpatterned n-doped "back side" and connected to arrays of charge amplifiers on the patterned p-doped "front side." (2) Special anti-reflective coatings that provide a high quantum efficiency for the scintillation light. (3) Custom integrated low-noise analog circuits (developed at LBNL) that provide a high density of electronics and close connections to the individual silicon photodiodes. (4) Custom "Winner Take All" circuit (developed at LBNL) that rapidly and continuously identifies among 64 amplifiers the amplifier with the highest pulse height. This is considerably faster than sequentially scanning the individual amplifiers. (5) The use of thin (< 1 mm) solid-state photodetectors rather than bulky (> 3 cm long) photomultiplier tubes to allow shorter imaging distances.

(Supported by DOD, Army Breast Cancer Research Program, Idea grant)

103. Positron 3D Imaging Instrument

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The primary objective of this project is the development of advanced detectors for the imaging of radio-labeled tracers in humans and animals with substantial improvements in spatial and temporal resolution. TASK I: To overcome the limitations in spatial resolution and maximum event rates that conventional PET tomographs have, we are developing a detector module consisting of a group of small, high density LSO (Lu_2SiO_5:Ce) scintillation crystals coupled on one end to a square phototube for timing information and coupled on the opposite end to an array of silicon photodiodes for position information. The immediate goal is the construction of a 29,000-crystal tomograph for imaging the human brain and animals with < 2 mm resolution in 3D. We are collaborating with a commercial U.S. positron tomograph manufacturer who will supply LSO crystals for detector modules of our design. During this project, we have developed the low-noise photodiode arrays and custom integrated charge amplifiers that can provide the signal-to-noise ratio necessary to measure the depth of interaction in the crystal to correct the off-axis radial blurring caused by parallax error. TASK II: To overcome the limitations in photoelectric stopping power, speed, and luminosity of existing scintillators for PET, we are developing new scintillators by (i) synthesizing and measuring a large number of pure and doped heavy-atom compounds to find those exhibiting fast x-ray induced luminescence, (ii) measuring the scintillation properties of optical crystals of promising compounds, and (iii) using existing quantum chemistry computer programs to guide the search for new scintillator materials. TASK III: To detect malignant breast tumors and distinguish them from benign densities and post-surgical scar tissue, we are developing both a compact PET tomograph and a compact gamma camera using the technology developed in TASK I. Adoption of these techniques and devices by other research institutions and industry will permit (1) the production of improved imaging instruments for the benefit of medical research throughout the world and (2) the reduction in cost and improvement in accuracy of clinical diagnostic imaging.

(Supported by DOE, Field Task Proposal)

104. PPG Project II: Advanced High Speed, High Resolution Positron Tomograph

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The overall objective of this project is to provide an advanced animal whole body/human brain positron tomograph for the medical science projects. This tomograph will make possible quantitative measurements of cardiovascular physiology in animal models by permitting high count rate dynamic studies with bolus injections of flow and metabolic tracers. It has the following advanced features: (1) lutetium orthosilicate (LSO) scintillators, which have 6 times greater light output and 8 times faster decay time than bismuth germanate (BGO); (2) photomultiplier tube readout to provide pulse height information (to reject scattered annihilation photons) and a timing resolution < 1 ns (to reject random backgrounds); (3) small (1.5–2.5 mm) crystals, read out individually by low-noise silicon photodiodes; (4) depth of interaction measurement for < 2 mm spatial resolution over the imaging volume; (5) small detector diameter of 35 cm for low noncollinearity error, high sensitivity, and reduced cost; and (6) full 3D mode over a subject port of 30 cm and an axial field of 15 cm (no inter-plane septa), for high sensitivity and a maximum noise equivalent event rate of > 700 k/s at 1 μCi/ml. We expect that U.S. Department of Energy funding will support purchase of the components needed for the 270 detector modules, that CTI PET Systems will provide the labor to assemble and test them, and that this project will provide the following: (1) construction, testing, and evaluation of pre-production detector modules; (2) assembling 270 pre-tested detector modules in a gantry at LBNL; (3) evaluating the completed high resolution

tomograph; and (4) developing attenuation measurement, background correction, and calibration procedures. This project will advance the technology further by developing time-of-flight capability and installing it in the high resolution LSO tomograph. Our goal is a timing resolution < 500 ps, which corresponds to < 7.5 cm. This improves the signal-to-noise ratio in the reconstructed 3D images by further reducing the random coincidence background and by localizing the events in 3D to reduce error propagation in the reconstruction process.

(Supported by NIH/NHLBI, P01 grant)

105. Search for Ultra-Fast, Heavy-Atom Scintillators

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The primary objective of this research is the discovery of new scintillators with very rapid emissions, and high atomic number, density, and luminosity. A scintillator with higher atomic number and luminosity than Lu_2SiO_5:Ce, and a decay time ≤ 1 ns would improve the detection efficiency, spatial resolution, and scatter rejection of positron tomographs and improve the accuracy of the reconstructed images by providing time-of-flight information. Additional objectives include the discovery of scintillators having higher stopping power and less dead time than NaI(Tl) for single photon tomography and having low afterglow and high light output at longer wavelengths (where silicon photodetectors have their highest efficiency) for x-ray computed tomography. During the course of this project, we have discovered the scintillators CeF_3 and $PbWO_4$ (which are in large-scale production for high energy physics), and $BaCl_2$, $LuAlO_3$:Ce, $LuBO_3$:Ce, whose crystal growth is under investigation. To accomplish these objectives, we will continue to use empirical and computational guidance in the selection of stoichiometric and doped compounds to synthesize and measure. We have built a pulsed x-ray source specifically designed to measure scintillation from powders as well as from crystal samples with high sensitivity and a timing resolution < 120 ps. We are using the DOE National Energy Research Supercomputing Center to perform first-principle quantum cluster calculations to improve the understanding of critical processes that enable or prohibit scintillation (hole transport, exciton formation, excitation of activator atoms, thermal quenching, etc.) and to develop computational methods for the efficient selection of compounds for synthesis and testing.

(Supported by NIH, R01 grant)

106. PET Imaging of Gene Therapy Delivery by Adeno-Associated Virus Vectors

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Lawrence Berkeley National Laboratory (LBNL) and Avigen Inc., an Alameda-based gene therapy company, will apply positron emission tomography (PET) technology to evaluate a novel gene therapy delivery system in the MPTP primate model of Parkinson's disease (PD), a disorder marked by dopamine deficiency. MPTP produces degeneration of dopamine cells in primates, a condition analogous to PD PD in humans. LBNL investigators are currently working with an imaging compound, [18F]fluoro-meta-tyrosine (FMT), which binds to cells that utilize the neurotransmitter dopamine and enables the quantitation of dopamine activity in the brain. New techniques developed by Avigen allow direct gene transfer to central nervous system (CNS) cells through the use of adeno-associated viral vectors (AAV). Gene therapy offers many advantages over traditional PD treatment by permitting manipulation of the dopamine synthesis pathway to increase CNS dopamine production. However, technical issues regarding the most efficient means of CNS gene delivery remain unresolved. AAV vectors for the delivery of dopamine decarboxylase (DDC), one of the enzymes required for the biosynthesis of dopamine, have been designed for PD treatment, but assessment of transgene expression is difficult in living animals. PET is used to monitor dopamine function over time

in MPTP primates after AAV gene therapy as an indicator of DDC transgene expression. AAVs have great potential for the treatment of PD, and LBNL's PET imaging techniques are effective means of monitoring successful AAV transfer of therapeutic genes for dopamine production.

(Supported by DOE and Avigen, small CRADA)

107. Breast Cancer Specific PET Instrumentation

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The overall aim of this project is to continue development of PET cameras whose geometry is optimized for detecting breast cancer or axillary node involvement. These post- x-ray mammography tools would determine whether suspicious structures observed in mammograms have the increased metabolism associated with breast cancers and can image the axilla to determine the extent of axillary node involvement. The instrumentation proposed has the potential to provide a cost effective, non-invasive alternative to biopsy as well as accurate information on axillary node involvement with significantly higher sensitivity than existing imaging techniques. The proposed technique relies on the fact that FDG is an excellent tracer for breast cancer with > 90% specificity and selectivity, as measured with conventional PET imaging. The proposed instruments consist of PET detector modules placed in close proximity to the breast (similar to a mammography unit) or the axilla (similar to a small diameter PET ring). These geometries improve the sensitivity and the spatial resolution significantly compared to a conventional PET camera (the sensitivity increase is a factor of 4–30 for the breast and a factor of 1–10 for the axilla), and so allow rapid identification of cancerous lesions and axillary involvement for structures down to 5 mm in size with a small (< 1 mCi) injected dose of FDG. The first funding period of this project focused on development of the base technology for a high performance PET detector module consisting of a large number of small LSO scintillator crystals, each coupled on one end to a photomultiplier tube (which provides a timing pulse and energy discrimination) and on the other end to an individual silicon photodiode (which identifies the crystal of interaction and measures the depth of interaction within the crystal). The present project focuses on construction of two PET cameras, development of reconstruction algorithms for these unique camera geometries, characterization of the imaging properties with phantoms, and preliminary evaluation of the imaging properties in patients. The small detector volume reduces the camera cost by a factor of 10 compared to a conventional PET camera. When combined with the lower amount of radio-pharmaceutical needed, this development could reduce the cost of a patient examination significantly. These cameras are designed specifically for breast cancer, but the detector module developed could also be incorporated into high resolution PET cameras with conventional geometries. These designs, when complete, will be offered to private industry to incorporate into a clinical instrument.

(Supported by NIH/NCI, R01 grant)

108. Radiochemistry for BNCT

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The overall goal of this project is the development of materials and procedures for the radiolabeling of boronated compounds with positron-emitting isotopes to assist in the synthesis and selection of new Boron Neutron Capture Therapy (BNCT)-agents. Specifically, we are proposing to construct targets for the production of ^{76}Br, ^{55}Co, and ^{124}I in the Center for Functional Imaging's (CFI) medical cyclotron located at LBNL and to develop the radiochemistry protocols for the attachment or insertion of these isotopes into boron complexes. The isotope production will involve modification of existing methodology currently in use for radioisotope production at CFI and development of thermal chromatographic techniques to purify the isotopes from starting materials. Radiochemistry techniques will include

procedures for the introduction of radioisotopes of halogens into carborane derivatives and the use of carborane complexes to chelate Co isotopes. Ultimately, radiolabeled analogs of potential BNCT-agents that are derived from these complexes will be used in in vivo and in vitro imaging studies with animal models to select the best BNCT-compounds and in human clinical studies to optimize clinical strategies for their use.

(Supported by DOE, Field Work Proposal)

109. Development of Tyrosine Kinase-Based Cancer Imaging Agents

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The goal of this project is to demonstrate and validate that labeled tyrosine kinase inhibitors can be used as imaging agents for tumors overexpressing these receptor associated enzymes. We have outlined a paradigm to develop unique positron and single photon emitting radiopharmaceuticals using the epidermal growth factor receptor tyrosine kinase (EGFRtk) and breast cancer as model systems. Many members of the receptor tyrosine kinase family are involved in tumor cell signal transduction pathways and are useful prognostic indicators. EGFR is overexpressed in 45% of breast tumors. Its prevalence has been correlated with poor responsiveness to hormonal therapy and poor patient prognosis. A series of aminoquinazoline derivatives, structurally similar to those known to inhibit EGFRtk at pico- to nano-molar concentration, suitable for labeling with fluorine-18, carbon-11, bromine-76 or iodine-123 will be synthesized. The ability to inhibit EGFRtk activity will be tested in an in vitro whole cell autophosphorylation assay. Specificity will be determined by testing the quinazolines ability to inhibit erbB-2 and erbB-3 tyrosine kinase activity. Potent inhibitors will be subsequently radiolabeled with no carrier added fluorine-18, carbon-11 or bromine-76 produced in our cyclotron or with iodine-125 from Amersham. The accumulation of the labeled tracers in tumor cells (MDA-435, MCF-7, MDA-231, MDA-468) possessing various levels of EGFR expression will be assessed in *in vitro* cell culture experiments; specific and non-specific binding will be measured. The amount of EGFR in the cell lines will be determined by a separate method and correlated with the uptake. The distribution and metabolic characteristics of the selected radiotracers will be evaluated in tumor-bearing mice. Tumor cells from the same cell lines used in the cell culture experiments will be implanted and grown in mice. Tracer distribution, efficacy and receptor-mediated uptake will be determined in the mouse model. Again, uptake in the tumors will be correlated with a direct measure of EGFR titer. Demonstration of the ability to visualize breast cancer with EGFRtk imaging agents will have dual impact. Not only will this expand our armamentarium of agents for the diagnosis and staging of breast lesions but it will also validate the use of tyrosine kinase-based imaging agents to target tumors in other tissues. Additionally, it will provide inroads into new areas of research in drug discovery and therapeutic intervention.

[Supported by NIH/NCI, R21 grant; State of Calif. (Univ. of Calif. Office of the President) Breast Cancer Research Program, Idea grant; and DOD Breast Cancer Research Program, Idea grant]

110. Experimental Medicine Development of Radionuclides

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Our goal is to develop synthetic methods for the incorporation of radionuclides into biochemical substrates and to evaluate the potential efficacy of these radiolabeled compounds in the study of physiological processes and mechanisms in both normal and diseased states. Our approach is to use readily available generator-produced (^{62}Cu, ^{68}Ga, ^{82}Rb, ^{122}I) and cyclotron-produced (^{11}C, ^{13}N, ^{15}O, ^{18}F, ^{64}Cu, ^{76}Br, ^{123}I, ^{124}I) isotopes, which possess desirable nuclear and chemical properties, as labels for novel radiotracers. The strength of our program lies in the focused development of radioligands, from isotope production and radiochemistry through the biological

evaluation of the tracers in humans, in order to answer significant medical questions. This program interfaces with the basic instrumentation (FTP 4450), medical application (FTP 4454) components of the Center for Functional Imaging. New efforts include the fabrication of target systems for our medical cyclotron to produce common and exotic isotopes for radiopharmaceuticals; the automation of the $^{122}Xe/^{122}I$ generator for the facile production of new cardiovascular radiotracers; the design of new targets for ^{18}F production by the (γ,n) reaction; the synthesis of PET tracers for the evaluation of steroid and growth factor receptor density in tumors; the determination of the scope and limitations of the reductive amination labeling chemistry; the application of the reductive amination strategy to label neuroreceptor agents, tumor agents and aptamers; and the production of neuroreceptor ligands for the α_2-adrenergic, acetylcholinergic, dopaminergic, sigma and vesamicol systems as well as mitochondrial and metabolic tracers to probe aging, Alzheimer's disease and Parkinson's disease. This program is the cornerstone of our specialized PET research training center.

(Supported by DOE, Field Task Proposal)

111. PPG Project IV: PET Radiopharmaceuticals for Metabolism and Flow

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The goals of this project are to use cyclotron produced and generator produced PET isotopes to label and evaluate novel tracers to measure parameters of cardiac physiology. ^{122}I (3.6 min. half-life) quaternary amines are attractive for rapid, repeat PET blood flow studies. ^{122}I will be produced from a $^{122}Xe/^{122}I$ generator system and used in rapid synthetic chemistry to produce the labeled quaternary amines. We will pursue the ^{125}I labeling of three classes of quaternary amines (phenyl trimethyl ammonium ion, iodobenzylguanidine and piperazinium ion) as potential cardiac flow agents and assess their feasibility for ^{122}I labeling as well as evaluate their potential imaging characteristics in the red blood cell perfused rabbit heart under normal, ischemic and hypoxic conditions. Promising tracers will be labeled with ^{122}I for in vivo imaging studies in project I. Additionally, we also intend to begin work on a fully automated generator/chemistry system relying on the latest in automation technology to show the viability of this generator as a suitable alternative for radiotracer production. A considerable body of evidence exists implicating mitochondrial loss or dysfunction in cardiomyopathies and normal aging processes of the heart. The loss of mitochondrial density and function in the heart has been correlated with mutations in the mitochondrial DNA. Studies in young and old rats have demonstrated that the loss of electron transport chain enzymatic function increases with age. Thus, a non-invasive imaging agent to measure mitochondrial density and function would greatly enhance our ability to define the loss of activity as well as contribute to the understanding of the pathophysiology of cardiomyopathies and aging. We propose to evaluate two classes of mitochondrial probes, rotenones and rhodamines, labeled with fluorine-18 and carbon-11. Specifically, we will study the uptake and retention of these tracers in isolated mitochondria and cultured liver cells. Rotenones: block the complex I with rotenone and measure uptake versus NADH and citrate synthetase activity. Rhodamines: measure uptake in whole cells versus media additives such as ouabain, CCCP and nigericin (modulators of membrane potentials). A similar set of experiments in the buffer perfused isolated rat heart will be performed. We will use the red blood cell perfused rabbit heart to define the tracer uptake and washout kinetics under normal, ischemic and hypoxic conditions. We will use an occlusion/reperfusion rabbit model to correlate tracer uptake with measures of mitochondrial density and function. The anticipated results from this project will be: i) the development of ^{122}I quaternary amines as flow tracers and the improvement of the ^{122}I chemistry with automation of the generator system, ii) the development of mitochondrial probes for the degenerative loss of electron transport function provided we can show that these agents are not merely extracted and retained as a function of flow.

(Supported by NIH/NHLBI, P01 grant)

112. Alzheimer's Disease as a Systemic Disorder

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This grant proposes to explore the hypothesis that Alzheimer's disease (AD) is a systemic disease caused by a chronic ATP deficiency resulting from diminished blood flow reactivity or defective glucose metabolism. Progressive damage to the nonregenerative central nervous system neurons is manifested early in the CNS because of the susceptibility of neurons to low blood flow and the limited ability of neurons to regenerate and to maintain mitochondrial enzymes. Specifically, we will investigate the role of systemic perfusion reactivity and systemic carbohydrate metabolism in AD. These hypotheses will be tested by experimental protocols including: (1) measurement of the deficit in acetylcholine neurons in AD by quantification of physostigmine stimulation of cerebral blood flow using high resolution PET with ^{122}I-HIPDM; (2) measurement of endothelium-dependent vasodilation of peripheral limb resistance vessels in AD patients in response to tourniquet-induced (blood pressure cuff) ischemia using a Doppler analysis method developed in previous work; (3) evaluation of kinetics of ^{18}F-fluorodeoxyglucose in exercising skeletal muscle of AD using positron emission tomography; (4) *in vivo* 4T magnetic resonance studies of oxidative phosphorylation potential in AD patients; (5) *in vitro* glucose metabolism studies using ^{14}C glucose in leukocytes; and (6) mitochondrial function assays of leukocytes in AD and controls. This proposal involves 284 different patients and subjects in 404 *in vivo* and *in vitro* non-invasive studies. The procedures are currently validated technologies using unique resources at Lawrence Berkeley National Laboratory. New critical experiments in this proposal are the method of performing *in vivo* studies of peripheral vessel relaxivity, the *in vivo* skeletal muscle metabolism studies using FDG-PET, and the *in vivo* skeletal muscle oxidative phosphorylation potential studies utilizing 4T magnetic resonance spectroscopy.

(Supported by pending NIH/NIA, R01 grant)

113. Compact Scintillation Camera for Medical Imaging

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We propose to develop a commercially viable line of compact nuclear medical imaging devices. LBNL has developed prototypes of several such devices based on scintillation crystal arrays coupled to photodiode arrays and controlled with a custom integrated circuit. Capintec would like to incorporate these core technologies (scintillation crystals, photodiode arrays, and custom integrated circuits) into a line of commercial products directed at nuclear oncology and cardiology, specifically 1) a miniature imaging probe for inter-operative detection of radionuclides to assist in cancer surgery, 2) a small compact camera for detection of thyroid disease, 3) two camera geometries for breast cancer imaging (appropriate for pre-surgical evaluation of breast cancer and nodal metastases), and 4) a larger camera for cardiac and other nuclear medicine studies. While the devices demonstrated by LBNL have successfully proved the feasibility of the detector concepts, the exact physical form requires modification before it can be incorporated into the above designs. For example, the pixel sizes and number of pixels (which affect the photodiode array and the scintillator crystal array) must be modified, more robust readout electronics must be developed, and the electro-mechanical interconnections and packaging must be miniaturized and ruggedized. The LBNL role in the collaboration is to provide the expertise in the critical core technologies and further develop the readout electronics, while Capintec will provide packaging design and technology, develop sources for the various components, and convert the prototypes developed in this project into marketable products. If successful, the project will provide DOE and LBNL with a licensee for technologies they have developed, possession of the prototype imagers for future research, and satisfaction from making research technologies available to the public. Capintec will obtain new, unique, high performance products that it can offer for sale. The nation will benefit from the

improved diagnostic accuracy (and hence quality of health care) made possible with these instruments.

(Supported by DOE and Capintec, Energy Research–Laboratory Technology Research CRADA)

114. LBNL Psychiatry Imaging Studies

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Under the scope of this work labor-intensive computerized imaging analysis of 30 three-dimensional PET and MRI studies which have already been acquired will be performed. In addition, PET studies of the brains of normal controls utilizing [F-18]-fluoro-meta-tyrosine will be performed throughout the year. This will allow us to determine rate constants of dopamine kinetics in different regions of the brain.

(Supported by pending State of Calif.–Dept. of Mental Health–Napa State Hospital, Research grant)

115. Sex Differences of Dopamine Metabolism

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The primary motivation behind this project is to use our special resources, and in particular our ability to measure non-invasively, in-vivo, human brain dopamine metabolism, in order to test for significant relationships between gender, sex-hormones, cognitive performance and dopamine metabolism in specific brain regions. We hope to gain insight regarding the possible protective effect of estrogen or estrogen-like substances in female patients with schizophrenia. This may have therapeutic implications. In addition, an *in vivo*, human, dopamine database, can be instrumental in other areas of brain research, where dopamine is playing a central role, such as in Parkinson's disease, Reinforced Learning Theory, Goal-Directed multi-task Motor Execution, Arousal Systems and Attention, and in Working Memory Systems. The current study is aimed at comparing regional cerebral dopamine uptake in healthy males and females utilizing the tracer [F-18]-6-fluoro-meta-tyrosine (FMT) and positron emission tomography (PET). As a part of the effort, dopamine (DA) uptake, differences will be tested in 15 healthy young adult males and 15 healthy young adult females. In addition, we will test for striatal DA uptake differences with ANOVA among 15 post-menopausal females who are receiving long-term standard hormonal replacement therapy (HRT), 15 post-menopausal females who are not, and 15 males age- and SES-matched to the older females. Estrogen, progesterone, and cortisol will also be drawn at the time of the FMT PET study of women in order to perform exploratory correlational analyses which will complement the group comparisons. In the males we will similarly obtain cortisol and testosterone at the time of the PET study. FMT is a relatively new compound for studying dopamine metabolism and it has certain advantages over the main alternative, [F-18]-fluorodopa, such as less problems with metabolites which may cause measurement errors. Sex differences have received considerable attention recently in schizophrenia. Schizophrenia studies have noted that the illness in females has a delayed onset of 4 to 6 years and during the first 10 years when estrogen levels are higher and the course of illness is milder than that seen for male patients. Furthermore, the course of illness in females deteriorates as they near menopause and estrogen levels decrease. These differences in schizophrenia, a disorder in which dopamine plays a prominent role, have led to interest in the molecular level mechanism of this sex difference and the interaction between estrogen and the dopamine systems.

(Supported by NIH/NIMH. R01 grant)

116. Choline-Based Imaging Agents for Non-Invasive Tumor Detection

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The overall aim of this project is to develop new radiopharmaceuticals for early detection of many forms of cancer of the lower abdomen, including prostate, colon, uterine and ovarian cancer, as well as improved delineation of tumors in the brain. ^{18}F labeled fluorodeoxyglucose (FDG) is fast becoming the imaging agent of choice for the detection of tumors and metastases using both Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) cameras. However, there are some practical limitations to using FDG to image all types of cancer. Most notably, FDG is excreted through the urine, and accumulation of activity in the bladder can obscure the detection of tumors and metastases in organs and tissues of the lower abdomen. FDG accumulates in normal brain tissue to nearly the same extent as tumor tissue, making brain tumor delineation difficult. FDG does not clear quickly from the blood and is taken up in inflammatory tissue confounding the scan. Recently, ^{11}C labeled choline was found to be an optimal imaging agent for many types of tumors. [^{11}C]choline clears the blood rapidly, it is not excreted through the bladder and is not taken up by normal brain tissue. However, [^{11}C]choline is not a clinically practical PET radiopharmaceutical because of the short carbon-11 half-life (20 min vs. 110 min for fluorine-18). FDG is widely available and distributed out of regional accelerator sites because of the 110 min half-life. With [^{11}C]choline, an on-site accelerator is necessary for access to this compound. Therefore, we propose to increase the availability of this interesting new tracer by screening analogs of choline labeled with fluorine-18, engineering the optimal tumor imaging agent. In this focused pilot and feasibility study we plan a four step approach. First, we will synthesize several fluorine containing analogs of choline. Choline is taken up in tumor cells and phosphorylated, the first step of phospholipid synthesis. This reaction is catalyzed by choline kinase. We have chosen our target compounds based on the known choline kinase specificity for choline analogs. Second, we will determine the substrate specificity of the choline analogs for choline kinase. We will perform an *in vitro* choline kinase assay and determine the rate of phosphorylation of the new choline analogs. This will be the first screen for these analogs. Third, those choline analogs that show retained biological activity in the choline kinase assay will be labeled with ^{18}F. Finally, the choline analogs will be evaluated *in vivo* in mature male rats. We will identify the distribution characteristics of these radiotracers especially in the brain, prostate and clearance organs, liver, kidney and the bladder. We will also study the *in vivo* metabolic characteristics of these new tracers. The predicted outcome of this project is a new longer lived imaging agent for the clear detection of cancer in the prostate and the brain. The translational potential of such an agent is increased by labeling with an isotope that allows the broadest possible distribution to the patient population. A network of distribution centers is already established for potential future commercial application of new tracers. Additionally, these compounds may be useful in magnetic resonance spectroscopy as fluorine-19 is a sensitive magnetic resonance nucleus.

(Supported by State of Calif. Cancer Research Program, Pilot and Feasibility Study)

117. Tumor Angiogenesis: Imaging and Therapeutic Intervention

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This proposal details the development and application of imaging agents to measure tumor angiogenesis utilizing tracers specific for the vascular endothelial growth factor receptor (VEGFR). Existing imaging techniques to measure metabolic and physiologic parameters relating to tumor angiogenesis following anti-angiogenic therapy with a neutralizing anti-VEGF monoclonal antibody will also be utilized in animal tumor models and pre-clinical human subject trials. Ultimately, VEGFR tracers will be

assessed as potentially superior markers of tumor angiogenic activity and its response to anti-angiogenic therapy. Our specific aims are: 1. To develop small molecule ligands/inhibitors of VEGFR-2 as vascular imaging agents and determine their potential to non-invasively quantitate tumor angiogenesis and its response to therapy with anti-angiogenic agents. Small molecule inhibitors (3-substituted indolinone derivatives) have been identified as specific ligands of VEGFR-2. These compounds will be developed for vascular receptor-specific PET imaging tracers that will be evaluated for their utility in quantifying tumor angiogenesis. 2. To refine existing vascular imaging strategies for the assessment of response to anti-angiogenic therapy with anti-VEGF monoclonal antibodies (Mab). In initial animal studies non-invasive assessments of vascular dependent metabolic parameters such as blood volume, permeability, glucose uptake, etc., will be performed. Tumor models responsive to inhibition with anti-VEGF monoclonal antibody and others resistant to this will be exploited to identify non-invasive parameters most suitable as potential predictors and/or surrogate endpoint of ultimate tumor responsiveness to anti-angiogenic treatment. Optimal imaging strategies will be incorporated into clinical trials of anti-VEGF Mab and compared with conventional measures of treatment response.

[Supported by pending Univ. of Calif. at San Francisco (NIH/NCI), R01 grant]

118. Biodistribution of New BNCT-Liposome-Complexes with PET

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The overall goals addressed in this proposal are the development of new boronated compounds that will accumulate in target tissues to facilitate treatment by Boron Neutron Capture Therapy (BNCT) and the development of PET-based imaging techniques and protocols that can be used for both compound selection and treatment planning. The potential applications of boron-enhanced neutron therapy include treatment of numerous cancers, including cancers of the brain, head and neck, prostate, colon, melanoma, cervical and breast, as well as non-cancerous inflammatory diseases, such as rheumatoid arthritis, which can be treated with BNC synovectomy. The specific aims for this project are: 1) the design and synthesis of new BNCT compounds, 2) the application of a new liposomal delivery system to enhance boron accumulation in the tumor cell, 3) the development of radiochemistry techniques for the radiolabeling of boronated compounds, and 4) the use of the radiolabeled BNCT compounds in the selection of the most promising BNCT agents. The new boronated compounds will be developed from two classes of biological molecules: polyamines and residualizing sugars. The synthetic polyamines will be selected on their bases of high DNA affinity and relatively low cytotoxicity. Cellobiose will be used as the disaccharide residualizing agent. Charged derivatives of carborane complexes (*nido*-carborates and fly-trap clusters) will be used for the boronation of these compounds. Techniques for radiolabeling the boron complexes will be developed for both commercially available (^{64}Cu, ^{67}Cu, ^{125}I) radionuclides and for isotopes produced on the LBNL biomedical cyclotron (^{18}F, ^{124}I, ^{55}Co,^{76}Br). Polymerized liposomes derived from polydiacetylene acids will be studied for their potential as delivery vectors for the specific transport of BNCT compounds to tumors. The surface of these liposomes will be modified to target specific receptors on the tumor surface (LDL, folate). The kinetics of the biodistribution of the BNCT compounds and liposomes will be studied with animal tumor models and human tissue cultures. Utilizing *in vitro* and *in vivo* radionuclide techniques, including Positron Emission Tomography (PET), the best candidates for future human trials will be selected.

(Supported by pending NIH/NCI, R01 grant)

119. Measurement of Fast Scintillator Materials for Radiation Detection

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Scintillation spectra and decays will be recorded for crystalline samples provided by LLNL using the pulsed x-ray facility available in the Center for Functional Imaging at LBNL. Results in the form of tabular and graphical data will be supplied to LLNL. Consultation with LLNL personnel about the interpretation of scintillation processes, prospective new fast scintillator materials, and locating sources of scintillator materials will also be provided as requested.

(Supported by LLNL/DOE, Integrated Contractor Order)

120. Ultrasound Mammography

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Researchers at the Lawrence Livermore National Laboratory are developing a prototype three-dimensional (3D) ultrasonic imaging system optimized for use in timely and cost effective breast cancer screening/mammography. Success of this project will be defined as the delivery of a working ultrasonic imaging system capable of finding lesions in human breast with equal or better efficacy compared to current x-ray mammography systems. LLNL has begun a multistage development of a totally new device for breast cancer screening. After the prototype device has been built, subsequent efforts are aimed at improving resolution of the system, increasing the speed of the procedure, reducing the system cost, and differentiating between benign and malignant lesions.

(Supported by Foundation)

121. OPUS: An Optically Parallel Ultrasound Sensor

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This development project addresses the need for a faster, less expensive method of transmission ultrasound. This imaging modality holds great promise for ultrasonic mammography. It utilizes the principle of frustrated total internal reflection to transduce acoustic pressure into optical modulation. These data can be acquired an entire 2D plane at a time, enabling fast acquisition of the data required for volumetric (CT) imaging with ultrasound.

(Supported by LLNL internal funding / DoD Army Breast Cancer Program)

122. Elastic-Scattering Spectroscopy for Noninvasive Diagnosis of Tissue Pathologies

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Often referred to as “optical biopsy,” optical spectroscopy of tissue can be performed through small fiber-optic probes, which can be mediated through endoscopes, to effect an instant, noninvasive diagnosis of the tissue condition. Our method is based on measuring the wavelength-dependence of elastic scattering of light by tissue, which changes with variations in the microscopic (cellular and subcellular) structure of the tissue. Preliminary clinical studies have demonstrated the potential of this method to diagnose malignant and pre-malignant changes in situ (in the bladder and various locations in the GI tract), and FDA trials are currently being initiated by a commercial partner, which has licensed the LANL patent. Current studies with medical collaborators are exploring the potential diagnostic applications of this method for breast cancer and other areas.

[Supported by U.S. Army Medical Research and Materiel Command (related fundamental studies sponsored by NIH)]

123. Time Resolved Photon Migration Tomography and Spectroscopy

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We are combining advanced illumination and time-resolved imaging strategies with computational models to develop optical methods for tomographic imaging and localized spectroscopy in scattering media. The sensitivity of light to physiological and biochemical parameters can provide information about the state and function of biological tissues not accessible to other techniques. This work exploits a novel photon counting imager developed at Los Alamos for remote imaging applications. The detector is coupled to a light collecting fiber-optic bundle detector system to measure the arrival time history and amplitude of the transmitted light emerging from many locations over the surface of the scattering medium. Time-resolved data will be used to reconstruct the absorption and scattering properties of tissues, using an iterative, model-based reconstruction procedure employing adjoint differentiation and gradient descent.

(Supported by Internal LANL, LDRD)

124. Vibrational Spectroscopy for the Identification and Detection of Molecular Changes Accompanying Carcinogenesis

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Vibrational spectroscopy has the potential to be a valuable tool for defining and subsequently noninvasively measuring molecular changes during the progression of cancer. It can be applied in a noninvasive fashion and is based on the intrinsic molecular features of biochemical compounds. The primary biochemical compounds in tissue are nucleic acids, proteins, DNA, lipids and in some cases a significant amount of carbohydrates. Each of these types of compounds has a unique vibrational spectrum. Therefore even in a complex biological system such as a cell or tissue, vibrational spectroscopies may be used to determine the ratios of concentrations of types of molecular species. Additionally, there are preliminary indications that the spectra of an ensemble of nucleic acid molecules (within cells or tissue) may be altered in the progression of cancer. It would be of considerable interest to have a detailed understanding of these changes. We are proposing to study molecular changes in well controlled multistep carcinogenesis models with which we have considerable experience using state-of-the-art vibrational spectroscopy and microscopy being developed at the Integrated Spectroscopy Laboratory at LANL.

(No current sponsors)

125. Noninvasive Monitoring of Regional Brain Function

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Brain function can be monitored externally and noninvasively by methods generally referred to as "photon migration." In the near-infrared spectral range, tissue, although highly scattering, has a low absorption coefficient, permitting scattered photons to travel several centimeters from an entrance point on the surface of the head, passing through the scalp, skull and several centimeters of brain tissue, and be detected at a location on the head surface a few centimeters from the illumination point. Our unique approach to this photon migration technique employs a broadband (white) light source, permitting the measurement of the brain hemoglobin spectra, among other things. Preliminary studies have shown localized, correlated changes in brain oxygenation with activation of different regions, such as the finger-motor cortex and the visual cortex.

(No current sponsors)

126. Time Resolved Optical Imaging of Neuronal Population Activity

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Optical imaging techniques can provide information on a microscopic level about the individual and collective behavior of neuronal populations. We are developing an advanced image probe and digital acquisition system designed for high performance functional neural imaging based on intrinsic light scattering signals. Two methods of reflectance mode illumination are being explored for fluorescence and polarized light measurements, and the system will incorporate an acousto-optic tunable filter to illuminate tissue with specific wavelengths for spectroscopic measurements. Our preliminary studies in the hippocampus and medulla have demonstrated several different optical changes associated with neural activation, including fast light scattering changes concurrent with swelling and electrical transmission, and slower changes in light absorbance associated with hemodynamic coupling to metabolic demand.

(Supported by Internal LANL, LDRD)

127. Computerized Tomography to Monitor Lung Tumor Therapy in Mice

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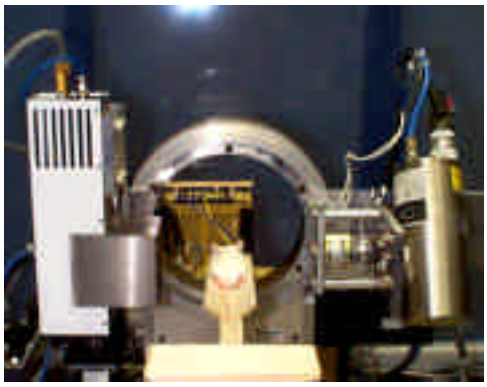
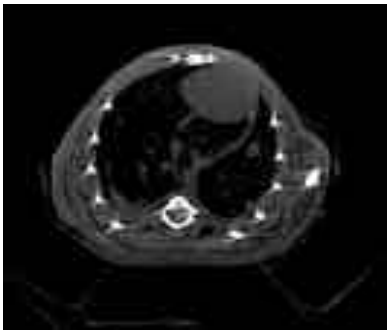
A new high-resolution x-ray computed tomography system has been developed at the Oak Ridge National Laboratory to study anatomic details of small animals *in vivo.* The prototype "MicroCAT" scanner has been used to study amyloid deposits in transgenic mice, fat distribution in obese mice, tumors and other anatomic abnormalities. The MicroCAT will be especially important for high throughput phenotypes screening in the ORNL Mammalian Genetics Research Facility. The instrument produces reconstructed images with spatial resolution approaching 50 microns. A high-resolution scan of a 1.5-inch section of a mouse is acquired in about 20 minutes, while lower resolution (screening) scans are acquired in about 7 minutes. At ORNL, we have developed a method for treating tumors in mouse lung by delivery of alpha particle emitting radioisotopes (^{213}Bi and ^{225}Ac) to vasculature serving the tumors. This procedure has been effective in curing small tumors in the lungs. The mechanism of tumor destruction is under study, and therapy of larger tumors is being attempted using radioisotopes with greater range in tissue (^{90}Y and ^{188}Re). It may be possible to follow the course of therapy of theses tumors in individual animals using CT. We have shown that animals anesthetized with avertine can be scanned in about 20 min. The scans show positive spots corresponding to positions of lung tumors of 1 to 2 mm in diameter, even though the animals are breathing during the scan. The procedure has been repeated at various times on the same animal over a period of several days, in effect, resulting in a tumor growth profile for individual lung tumors. We are currently assessing the sensitivity of detection of this technique while animals are being treated with the radioimmunotherapy. Successful visualization of



(a)



(b)

(a) MicroCAT hardware and (b) image of a tumor in a mouse lung.

tumor regression may promote hypothesis driven studies on the mechanism and allow rapid evaluation of several different treatment modalities.

(Supported by LDRD)

128. Dual Modality Small Animal Imaging

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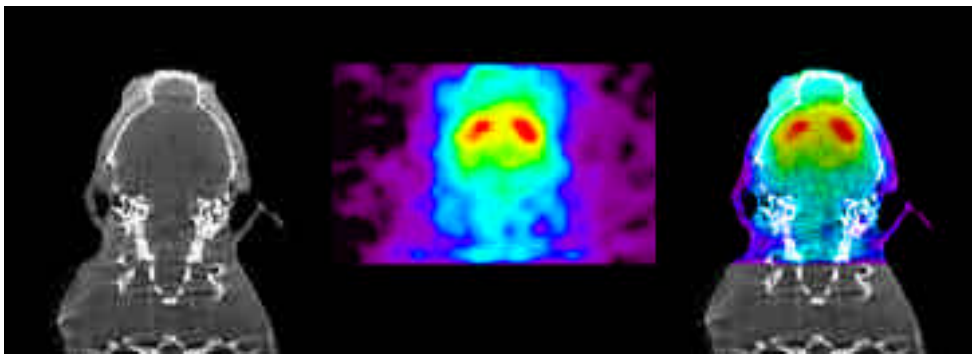
As our understanding of the function of molecules regulating biochemical pathways improves, nuclear medicine is becoming an important aid in the study of biochemical processes. Small animal specific nuclear medicine systems, particularly small animal positron emission tomography (PET) systems, have recently emerged as valuable tools for identifying biochemical dysfunction in small animal models as well as a means for monitoring therapeutic progress. While these new PET imaging systems offer impressive spatial resolution (< 2 mm FWHM) compared with their clinical counterparts (typically ~4 mm FWHM) this resolution is poor compared with the small size of the animal models. For example, a mouse is approximately 20 times smaller than a human patient, implying that the *relative* resolution of a small animal PET scanner is approximately a factor of 10 poorer than that of a human scanner. To compensate for this loss of relative resolution, it is important to combine the new small animal PET technology with a high-resolution anatomic imaging system. ORNL has recently developed a small animal specific x-ray computed tomography (MicroCAT) system with approximately 50-micron resolution. We propose the development of a new dual modality PET/CT system using the MicroCAT gantry as the foundation (Figure 1). By acquiring CT and PET data in the same session, fully registered data sets may be obtained and merged, providing a previously unavailable combination of functional and anatomic data. Furthermore, the availability of a high-resolution anatomic data set will enhance scatter and attenuation correction of the PET data set and provide a valuable guide for iterative PET image reconstruction. As an example of the image data that may be obtained with a dual modality system, the figure shows an overlay of a mouse CT image with a mouse PET image. The PET data provides functional information unattainable with anatomic imaging systems while the CT data provides an anatomic map against which the functional data may be observed.

(Supported by LDRD)



(a) (b) (c)

Figure 1. Comparison of (a) an ORNL MicroCAT image, (b) a UCLA microPET image obtained using the cocaine analog ^{11}C-WIN 35,428 which binds to the dopamine transporter in the mouse, and (c) an overlay of the CT and PET images. (The microPET image was provided by the UCLA Crump Institute for Biological Imaging).

129. Photoacoustic and Ultrasonic and Photoacoustic Detection for Non-invasive Medical Diagnosis

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Although various technologies are becoming available to detect cancer, there is no instrumentation system that provides sensitivity, portability, and ease-of-use for small clinics or physician's offices remote from large medical laboratory settings. The main emphasis of this research proposal is on the development of medical diagnostic instrumentation for rapid and early detection of disease such as cancer of organs that are not easily accessible using endoscopes (e.g., breast and brain monitoring). The proposed technical approach involves the development of a novel non-invasive method and instrument for improved medical diagnosis using a new approach based on photoacoustic and ultrasonic (PACEUS) detection. The unique combination of both ultrasonic and laser photoacoustic methods, which is aimed at providing physical as well as spectrochemical properties of biological constituents of tissues, will significantly extend the usefulness of non-invasive medical diagnosis. The proposed research project involves several unique features. Conventional ultrasonic and photoacoustic methods have been developed and used previously. However, to our knowledge, the proposed PACEUS technology combining ultrasound and laser optical/photoacoustic techniques has not yet been applied to biomedical monitoring. The PACEUS device is designed to be easy for use at small clinics or physician's offices. This project will lead to improved, rapid and cost-effective medical diagnosis of diseases (e.g., breast cancer, prostate cancer, stomach and brain injury).

(Supported by DOD, other WFO)

130. Surface-Enhanced Raman Medical Diagnostics (SERMED)

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Vibrational spectroscopies are important techniques for chemical and biological analysis due to the wealth of information on molecular structures, surface processes, interface reactions that can be extracted from experimental data. Especially, Raman spectroscopy has recently enjoyed a renewed interest among researchers in many fields because of earlier observations of enormous Raman enhancement for molecules adsorbed on microstructured metal surfaces. This increase in Raman signal is the result of a surface enhancement process, hence the term surface-enhanced Raman scattering (SERS) effect. The observed Raman scattering signals for the adsorbed molecules are found to be more than a million times larger than those expected from gas-phase molecules or from non-adsorbed compounds. These enormous enhancement factors, which help compensate for the normally weak Raman scattering process, open new horizons to the Raman technique for medical diagnosis. We have recently developed a novel technology based on SERS detection for medical diagnostics (two US patents pending). The technique, referred to as SERMED, provides the drastic enhancement in the Raman signals of tissues and biological samples, which allow sensitive detection of subtle changes of trace bioconstituents for biomedical diagnosis. The proposed SERMED technique and probes will allow rapid in vivo and in situ analysis and can be used for (1) non-invasive biomedical diagnosis directly at clinical offices and (2) rapid drug testing in the workplace.

(Supported by OBER)

131. Development of New Diagnostic and Therapeutic Radiopharmaceuticals for Nuclear Medicine

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Research in the ORNL Nuclear Medicine Program is focused on the development of new tissue-specific radiopharmaceutical for diagnostic and therapeutic applications in nuclear medicine. Key strengths are in the areas of radiopharmaceutical chemistry, biochemistry, animal evaluation and radiochemistry. Research is focused on the design, development and animal testing of new tissue-specific radiopharmaceutical for in vivo nuclear imaging diagnostic applications in molecular nuclear medicine, to evaluate brain chemistry, head function and tumor localization, and for various therapeutic applications, including cancer treatment

New receptor-specific ligands have been developed and specific molecular conformations synthesized which are specific for the cerebral and myocardial muscarinic-cholinergic receptors. The ORNL developed iodine-123-IQNP and fluorine-18-FQNPe agents have been developed for the monitoring muscarinic receptor changes in dementias. These receptors are of particular importance, since their changes occur in many diseases, such as in memory loss, mood disorders and cardiac function. Alterations in the cerebral muscarinic-cholinergic system also occur in Alzheimer's disease. These new ORNL agents radiolabeled with iodine-123 for SPECT and carbon-11 or fluorine-18 for PET hold promise for noninvasive in vivo imaging of changes in receptor activity which occur in various dementias. Detailed animal studies, including specific receptor-blocking experiments, have demonstrated the selectivity and relative specificity of the iodine-123 agents for the muscarinic receptor, and SPECT studies in monkeys demonstrated the high uptake in receptor-rich cerebral regions. The development of the technetium-99m analogues for more broad use is also being pursued. In the area of heart imaging agents, the isomers of the iodine-123-BIMPP cardiac imaging agent developed at ORNL for evaluation of myocardial viability have been resolved and evaluated. For the evaluation of pancreatic insufficiency, the iodine-131-MIPAG triglyceride agent has been developed as a simple alternative to determine excreted urinary activity as a measure of pancreatic function. For cancer treatment, a variety of rhenium-188-labeled agents have been prepared and are being evaluated.

Several patents have been issued for new technologies developed at ORNL. Various new agents are evaluated in collaborative programs with over 20 Medical Cooperative Programs at clinics, universities, and other research institutions in the United States, Europe, Australia and Asia. Collaboration with external organizations and technology transfer are important activities that bridge the gap between development and testing of new agents developed at ORNL and use at other institutions. Several collaborative clinical programs through physician-sponsored IND's are evaluating bone pain treatment with the rhenium-188-labeled HEDP and Re(V)-DMSA agents which target skeletal metastases in patents presenting with metastases from various cancers. Primate studies are in progress and the first clinical SPECT studies with our iodine-123-labeled IQNP by collaborators are expected to begin in early 1999. Protocols are being developed in conjunction with the IAEA for use of rhenium-188 particles for hepatic cancer. Our BMIPP cardiac imaging agent has been commercialized in Japan. The ORNL Nuclear Medicine Program has also traditionally offered research opportunities for graduate and undergraduate students, postdoctoral fellows and guest scientists.

[Supported by OBER, NE (in the past), and CRADAs]

132. Evaluation of Agents for Vascular Brachytherapy for the Inhibition of Arterial Restenosis

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Because coronary vessel restenosis is a major problem encountered in 30-40% of the greater than 500,000 percutanous transluminal coronary angioplasty (PTCA) procedures performed in the U.S. annually, the evaluation of new methods for inhibition of the hyperplastic component of restenosis is of major importance. The only effective method yet

identified is the use of radiation. Various methods for introducing radioisotopes into the vessel are being evaluated. Investigators in the ORNL Nuclear Medicine Program envisioned and pioneered the use of liquid filled balloons using the rhenium-188 radioisotope for this purpose. Important benefits of using rhenium-188 for the liquid-filled balloon approach for vessel irradiation in contrast to other therapeutic radioisotopes are the uniform dose delivery to the vessel wall and the anticipated very low costs per unit-dose, contributing to a reduction of health care costs.

This major research area in the ORNL Nuclear Medicine Program is focused on the development of new therapeutic vehicles for vascular brachytherapy, involving the inhibition of arterial restenosis following high pressure balloon angioplasty (PTA). A clinical prototype alumina-based tungsten-188/rhenium-188 generator has been developed and optimized for clinical use. Current research at ORNL involves evaluation in animals of the dosimetric consequences of balloon rupture. The synthesis and evaluation of various carrier/complexation agents radiolabeled with rhenium-188 is being developed, including rhenium-188-(V)-MAG3, -DTPA, etc. The biodistribution and excretion kinetics of these species as well as rhonium-188 perrhenate are being evaluated in laboratory animals, and the biokinetic data then used for dosimetry estimates to predict the organ dose values in the worst case scenario of balloon rupture. Similar studies using other reactor-produced high beta energy-emitting radioisotopes are in progress.

Patents have been issued for the ORNL methods for concentration of solutions of rhenium-188. The process for rhenium-188 concentration was exclusively licensed to Mallinckrodt Medical in 1997. Several physician-sponsored clinical protocols are evaluating the use of the rhenium-188 agents for inhibition of arterial restenosis after balloon angioplasty. Protocols have been developed with collaborators at Columbia University and the FDA-approved Phase I studies were initiated in September 1997 with now rhenium-188 agents for restenosis therapy. Other collaborative projects exploring the use of rhenium-188 are supported by Cooperative Research and Development Agreements (CRADA), which have been established with Mallinckrodt Medical and InnerDyne, Inc., to further explore the use of rhenium-188 and other therapeutic radioisotopes with liquids and labeled-stents for this introvascular brachytherapy application. In conjunction with Investigators at the Oak Ridge Associated Universities (ORAU), a new Monte Carlo-based program has been developed for dose phoning for vascular brachytherapy.

[Supported by OBER, NE (in the past), and CRADAs]

133. MUSTPAC – Medical Ultrasound, Three-Dimensional and Portable with Advanced Communications

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Two innovations provide the first effective capability to perform ultrasound exams outside of traditional clinical settings. These are: 1) a data acquisition system that removes the need for an expert to acquire ultrasound data, and 2) a "virtual ultrasound probe" that provides the diagnostician with a familiar user interface with which to navigate the data. The image data can be acquired at a remote location and transmitted to a hospital for interpretation. A video image, transmitted with the ultrasound data, provides additional diagnostic information. A first generation system was field tested in Bosnia in August 1996. A Discover Award was received in late May 1997. The system was part of a U.S. Mt. Everest Extreme Expedition in May of 1998. Images were acquired and sent via satellite to PNNL and then routed to doctors at Yale University and Walter Reed Army Hospital. A prototype system is currently at Georgetown University where the co-inventor, Army Major Chris Macedonia, M.D., is currently on rotation. More information is available at http://www.pnl.gov/3dmed

(Supported by Defense Advanced Research Projects Agency)

134. Highly Uniform Transverse Magnetic Field Systems for Medical Magnetic Resonance Imaging (MRI) Applications

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A new calculation method of decomposition of field variables in azimuthal harmonics in two dimensions has produced a method of locating current-carrying wires that will produce magnetic fields whose uniformity is limited only by the accuracy of construction. Field homogeneities of better than 1 part per million should be readily achieved with ordinary construction techniques. Unlike the field of the typical solenoid used for MRI, the field is perpendicular to the axis of the system. A relatively small number of conductors will produce highly uniform fields, allowing a very open magnet to be built for access to the patient and for patient comfort. Also unlike solenoids, the field can be contained entirely within the magnet without shielding, for increased safety and reduced space requirements. The system has very low inductance, which means that the magnet can be brought to full field strength very quickly compared with a solenoid. This will permit a low-duty-cycle device to be built that can do rapid high-resolution real-time imaging at high fields without the need for expensive superconductive systems. The calculation method also produces novel single-sided uniform field configurations, with wires arranged on a half-cylinder for completely open access on one side. An MRI system based on this field configuration combined with rapid imaging techniques could reduce costs of MRI imaging by reducing magnet and site costs without the loss of image quality associated with low-field magnets. The open configuration also reduces patient claustrophobia, and the highly effective containment of the field within the magnet makes location very flexible. The system scales readily to small devices for specialized applications. No current work.

(Supported by internal funds)

135. Development of Large-Area Cadmium Zinc Telluride Imaging Arrays for Nuclear Medicine

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Unlike conventional gamma-ray cameras, this new imaging system can be installed in small breast cancer clinics, where their ability to image the breast from different angles could reduce the dependence on biopsies. These compact cameras could also be placed in hospital emergency rooms to speed diagnosis of heart attacks. The principles of operation involve a cadmium zinc telluride crystal that generates electrical signals when excited by x-rays or gamma rays. The crystals are able to read the current directly without aid from converters. The result is sharper images on the computer screen. Hospitals will spend about $540 million on gamma-ray cameras this year. This new gamma camera is expected to expand the boundaries of nuclear medicine as the 50-pound imaging device replaces the 3,000-pound cameras in current use. Time Frame: FY97 and ongoing.

[Supported by Digirad Corporation (CRADA)]

136. Innovative Low-Dose X-Ray Imaging Solutions

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Cardiac Mariners and Sandia are developing solid-state detectors for use in medical imaging (fluoroscopy) applications. These systems, because of the unique detector technology, improve the quality of X-ray images by reducing scatter, dramatically reduce radiation to the patient, physician, and staff; and provide continuous operation and lower cost of use. By utilizing cadmium zinc telluride as the sensor material, the system can generate X-ray beams, measure them, store the information and generate

output values while eliminating scatter which degrades contrast and resolution. In addition, the system is totally digital, unlike conventional systems. The system will provide clear advantages to both the doctor and the patient while reducing operating costs and X-ray exposure and increasing system flexibility and efficiency. FY99 (New project).

(Supported by Cardiac Mariners and Technology Partnerships Program)

137. Virus Structural Studies Using Low-Angle Single Crystal Diffraction and Time-Resolved Solution Scattering

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We have developed the low-angle single crystal diffraction technique to help solve the phase problem in virus crystallography. This technique also allows visualization of loosely ordered structures, which are often averaged out in high-resolution studies, such as nucleic acids in virus capsids. We are studying how nucleic acid interacts with capsid proteins to obtain general understanding of virus assemblies. Studies of this kind will eventually help develop medicines that perturb the virus assembly processes.

(Supported by NIH)

138. Small-Angle X-Ray Scattering Imaging of Cancer Tissues

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Work is in progress to develop an imaging technique that uses x-ray scattering to distinguish cancer tissues from healthy ones. Transmission-mode x-ray imaging techniques are currently employed to detect cancer. X-ray absorption does not, however, directly reflect structural features of cancer cells that are likely to exist in sub-cellular level in earlier stages of cancer development. By monitoring x-ray scattering from tissues we intend to monitor abnormal growth of biological fibers such as collagen and hardening of tissues.

(Supported by NIH)

139. Fe XAS K-Edge Studies of the Non-Heme Iron Enzyme Phenylalanine Hydroxylase

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Phenylalanine hydroxylase is a mammalian amino acid hydroxylase that initiates the detoxifications of high levels of phenylalanine from blood. Dysfunction of this enzyme causes irreversible, progressive brain damage that results from buildup of phenylalanine and its neurotoxic metabolites. The active site contains a non-heme iron center, for which its reduced state is the catalytically important state. X-ray absorption spectroscopy is used to characterize the structure of this site, both in the presence and absence of substrates and competitive inhibitors of the substrates. An understanding of the structures will aid in elucidating the complex mechanism of phenylalanine hydroxylase catalysis.

(Supported by NIH, NSF, and DOE)

140. Resolution Improvements in Electron Microscopy of Biological Assemblies Using X-ray Scattering Amplitudes

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We have developed a technique to improve cryo-electron microscope image reconstruction for structural studies of icosahedral virus particles and fibrous biological assemblies. Some of the features of reconstructed structures are incorrect if proper corrections in electron microscopy are not made. Due to a number of instrumental restrictions the corrections can be very difficult. The use of solution x-ray scattering amplitudes makes it straightforward to make the corrections. We are studying herpes simplex virus particles, actin bundles from horseshoe crab sperm cells, and bacteriophage particles.

(Supported by NIH, DOE)

141. Structural Studies of Myelin Glycoproteins by Solution X-Ray Scattering

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Myelin sheath is the major component of the nerve network. We are studying structures of glycoproteins P0 and PAS, which constitute a large fraction of the entire protein population in myelin. Mutations of the P0 protein, which are likely to disrupt its structural characteristics, cause diseases such as Dejerine-Sottas syndrome (DSS), a severe demyelinating peripheral neuropathy with onset in infancy.

(Supported by NIH, DOE)

142. Time-Resolved Muscle Fiber Diffraction

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Frog skeletal muscle fibers are being studied to understand mechanism of muscle contraction. We are specifically looking at potential structural changes in the direction perpendicular to the long axis of the fiber, though much of modern structural studies are focused on those in the fiber axis. We believe that elasticity of the fiber structure plays an important role in contraction, and the elasticity of the structure that holds the thick and thin filaments must be studied.

(Supported by NIH, DOE)

143. High Resolution Imaging of Gene Expression in Live Animals

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The Jefferson Lab Detector Group is collaborating with the Department of Biology at the College of William and Mary (Dr. Margaret Saha, PI), and the UCLA Crump Institute of Biological Imaging (Dr. Simon Cherry, co-PI) to develop a high resolution gamma imager for small animals. A prototype detector has been tested at the Department of Biology of the College of William and Mary and is being optimized for detection of the gamma radiation of ^{125}I (Iodine-125). Iodine-125 is the isotope that is used with certain molecular biology techniques to probe for particular gene products in a live animal. The ability to image gene expression *in vivo* (in live animals) will provide a valuable tool for molecular biology and human disease research. Current gene expression techniques take *in vitro* snapshots of the

state of expression of the gene of interest. In order to get an actual measurement of the animal's state of expression of the gene of interest, for instance, in a brain, the animal's life must be terminated. Other examples of applications of small animal gamma imagers include studies of human cancers, such as cancers of prostate and breast, in animal models. The Detector Group also recently installed a small version of the ^{125}I imager in the Weizmann Institute, Rehovot, Israel (Dr. Amos Breskin, PI), and a small imager to image Tc-99m uptake at the University of Athens, Greece (Dr. Nikos Giokaris, PI). In both cases the imagers will be used in cancer research using mice.

(Supported by The National Science Foundation)

144. A Compact, High Resolution Scintimammography Camera

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The Detector Group is currently collaborating with Dilon Technologies, Newport News, VA and Johns Hopkins University (Dr. Cahid Civelek, PI) on a medical instrumentation project to improve scintimammography—a nuclear medicine method of breast tumor detection. Scintimammography uses standard radiopharmaceuticals to locate the tumor. Radiopharmaceuticals are specially prepared chemicals which carry a gamma-ray emitting radioactive isotope and are markers to certain biological processes. Medical researchers have shown that several types of cancer cells uptake and accumulate these markers more readily than normal cells. The Detector Group has built several prototype economical portable gamma cameras that show high potential for better tumor detection in the breast than the most advanced standard medical imaging instruments currently available. Unlike standard devices, this imaging detector is capable of capturing enough close views of the tumor to increase accuracy in detection and localization of small lesions. The latest gamma imager prototype is undergoing clinical trials at Johns Hopkins University, Baltimore, MD. One patent was granted and one more has been submitted for the technology we have developed.

(Supported via CRADA between Dilon Technologies and Jefferson Lab, sponsor is HENP, Energy Research, Office of Science)

145. A Compact Beta/Gamma Probe to Assist Tumor Surgery

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The Detector Group is collaborating with Hampton University (Dr. Cynthia Keppel, PI), Duke University (Dr. Martin Tornai, co-PI), and East Carolina Medical School (Dr. Laureen Tafra, co-PI) on a project to improve pre- and intra-operative detection of cancerous lesions before and during surgeries by using special imaging and non-imaging radiation probes. The project is supported by an NSF grant to Hampton University (Dr. Cynthia Keppel, PI). Prior to surgery to remove a cancerous lesion, the patient is injected with a radiopharmaceutical. The higher metabolic rate of the cancer increases the uptake of the radiopharmaceutical. During surgical procedures to remove a malignant tumor, the probe is used to identify tissue that has metabolized more radiopharmaceutical than neighboring tissue. (Visually, one cannot distinguish between tissue that is cancerous and tissue that is healthy.) Thus, the surgeon has greater confidence that all of the cancerous tissue has been surgically removed. The Detector Group built several intra-operative prototypes of positron probes to be used with the known oncological agent, fluoro-deoxy-glucose (FDG), which is glucose labeled with a ^{18}F positron emitter. The latest beta (positron) probe prototype, which is based on a single crystal scintillator and a small photomultiplier, was successfully used in a melanoma cancer removal on October 14 and 28 in East Carolina Medical School by surgeon Dr. Laureen Tafra. The standard gamma probe failed to localize the lesion. The concept of the beta/gamma probe designed in the Detector Group was submitted for a patent. Imaging probe prototypes are also under development and a portable PET imager was successfully tried in

the surgery room before and during the operation on October 28 to better localize the lesion. (This pioneering surgery was recorded by two TV stations from Greenville, NC, and was shown nationally on many TV stations in November.)

(Supported by Hampton University and HENP, Office of Science)

146. Assisting Breast Tumor Biopsy with Positron Emission Mammography (PEM)

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The Detector Group is collaborating with West Virginia University (Dr. Ray Raylman, PI) on a project to improve the accuracy of core breast biopsy by adding a system of two compact positron emission tomography detectors to assist digital mammography in breast tumor localization. The Detector Group built two prototypes of positron emission mammography imagers to be used in this application. The latest PEM imager prototype based on a photomultiplier array will be installed before the end of this year at WVU to undergo clinical evaluations.

(Supported by HENP, Office of Science, and West Virginia University)

147. High Rate Gamma Camera

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The proposed device is a very compact, high rate, high resolution gamma imaging camera that will be attached to the patient's chest and can accurately image radiopharmaceutically tagged blood when it makes its first pass through an exercised (patient is on a treadmill) cardiac system. We anticipate that this will permit the functionality of heart operation in normal conditions and under stress to be assessed much more precisely than is presently possible. Bulky and/or slow devices available on the market are not capable of this unique imaging of the patient's heart with automatic correction for the patient's movement.

(Under a proposed Laboratory Technology Research (LTR) Grant, a CRADA will be executed between Jefferson Lab and Dilon Technologies. Sponsor is Office of Computational Technology Research, Office of Science)

Informatics and Mathematical Modeling

Informatics in biomedical research broadly refers to applications of computer technology in biomedicine, including clinical information systems, networking, image processing and display, and related activities. Mathematical modeling is an analytical tool for understanding complex data sets and biologic phenomena which has a powerful predictive role, especially in areas that are not readily approached experimentally or ethically.

148. Interspecies Extrapolation for Radiation-Induced Carcinogenic Risk

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Argonne research has demonstrated that age-specific death rates, associated with endogenous causes of death, possess an intrinsic mortality signature. Using a scaling approach developed by the Laboratory to adjust for differences in life span between species, Argonne has demonstrated that the intrinsic mortality signatures of mice, dogs, and humans are statistically indistinguishable. In other words, scientists can predict age-specific mortality in humans from the mortality experiences of laboratory animals. In a nation where the health consequences of population aging have huge financial implications (e.g., Social Security and Medicare), this finding is of tremendous significance.

Interspecies prediction models for population aging have also been generalized to predict health effects in populations exposed to radiation. Radiation-induced mortality among beagles exposed continuously to ^{60}Co λ rays for the duration of life has been predicted successfully from a dose-response model for laboratory mice similarly exposed. Preliminary analyses suggest that age-specific mortality among the survivors of Hiroshima and Nagasaki can also be predicted from a dose-response model derived for mice receiving a single exposure to ^{60}Co λ rays. The Laboratory's research proves that data collected from studies involving laboratory animals is extremely relevant to the prediction of human health effects. The Argonne database for laboratory animals is a unique and a significantly valuable national resource for addressing radiobiological issues related to human exposure to radiation (e.g., NASA's health concerns for human crews on deep space missions).

(Supported by DOE, Office of Biological and Environmental Research)

149. Studies of the Activities of ALS Mutant SODs Using Pulse Radiolysis Techniques

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The link between familial amyotropic lateral sclerosis and mutations to the enzyme superoxide dismutase is, with the production of transgenic mice containing a mutant enzyme and demonstrating the symptoms of ALS, quite conclusive. The in vivo function of superoxide dismutase is to convert superoxide radicals to oxygen and hydrogen peroxide. Understanding the effect of these mutations on the function of the enzyme is integral to understanding the mechanism of the disease and devising treatments. The focus of the work proposed in this project involves collaborative studies of the mechanism by which these mutated enzymes dismutate the superoxide radical. The use of fast kinetic techniques at our disposal allows measurement of the rate constants under a wide variety of experimental conditions. As the research to date suggests that these enzymes are quite active and that the deleterious effects observed in vivo are related to a gain-of-function in the mutated enzymes, the mechanism may involve a process that is triggered under specialized conditions (temperature, pH, ionic strength, the presence of specific substrates). Studies of enzymatic activity and aspects of the enzymatic mechanism under differing experimental conditions that can serve to mimic

various aspects of in vivo chemistry will, therefore, serve to shed light on the enzymatic dysfunction.

[Supported by ALS (Lou Gehrig's Disease) Association]

150. Microdosimetric Characterization of Clinical Neutron Beams

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The Neutron Therapy Facility (NTF) is developing the capability to perform microsdosimetric characterizations of clinical neutron beams. The system uses linear energy transfer (LET) proportional counters and a readout system based on the one developed at the University of Wisconsin in Madison. The therapeutic quality of clinical neutron beams is affected by collimating and beam shaping materials. At present no two clinical neutron beams in the world are exactly the same and there is a dearth of data describing the effects of different materials used in the beams. Microdosimetric characterization of beams is important for achieving uniform treatment outcomes at different facilities by minimizing materials that degrade beam quality.

(Supported by internal funds)

151. Adaptation of PEREGRINE Software for Clinical Neutron Beams

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PEREGRINE is a computer code used to calculate dose distributions in patients receiving conventional photon radiotherapy. It was developed at Lawrence Livermore National Laboratory using previously classified cross sections and is now licensed for sale in the open market. Benchmark tests indicate that its calculations are superior to any other commercially available a software for photon patient planning. In collaboration with the University of Wisconsin we have done preliminary measurements to acquire the beam data necessary adapt PEREGRINE for our clinical neutron beam, but more software work is needed to make the code clinically useful for neutron therapy. Use of PEREGRINE would enable us to directly transfer defense technology to improving the quality of care for patients. It might also throw some light on the differences in the effectiveness of neutron and photons in the treatment of cancer.

(Supported by internal funds)

152. Full Body Human Surface Profiling

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The INEEL acted as program managers and technical consultants on this project for the air force. Currently, we have no intellectual property and the applications of the system developed are being pursued elsewhere at government and commercial sites. The technique has substantial applications in the medical arena, as evidenced by the recent special report on the Discover channel regarding the use of the equipment at WPAFB to measure and manufacture close fitting masks to control facial scarring in burn victims. This is a collaborative effort.

(No current sponsor)

153. Boron Neutron Capture Therapy (BNCT) Research Program

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The Idaho National Engineering and Environmental Laboratory (INEEL) has a long-standing history in basic nuclear science research and in non-reactor applications of the nuclear and radiological sciences. One aspect of this is that we have established, in collaboration with other DOE laboratories as well as

several major domestic and foreign universities and other institutions, a well-recognized record of continuing contributions to research progress in the fields of fast-neutron external-beam radiotherapy and epithermal-neutron boron neutron capture therapy for cancer. The INEEL conducts research and supporting technology development in the fields of computational and experimental neutron biophysics and dosimetry, accelerator- and reactor-based neutron source development and deployment, chemical synthesis of advanced boron neutron capture agents, as well as high-precision chemical analysis of boron uptake in tissue. Summaries of specific major activities and accomplishments during 1998 are given below. We continued to support the human trials of epithermal-neutron BNCT for glioblastoma that are ongoing at Brookhaven National Laboratory. This involved development and maintenance of computational dosimetry and treatment-planning software for use at Brookhaven as well as validation of treatment plans for each patient entered into the Brookhaven experimental protocol. A major new version of the INEEL BNCT treatment planning software (SERA - Simulation Environment for Radiotherapy Applications) was completed and released for beta testing at Brookhaven during the year. This new software package offers significantly (factor of 6 to 8) faster execution times as well as much-improved capabilities for medical image handling and display, image reconstruction, and dose display. It is expected to replace earlier INEEL developed software for treatment planning (BNCT_rtpe) that has been in use since the Brookhaven trials began in late 1994. It will also be licensed for use at other domestic and foreign BNCT research institutions. INEEL is also a party to a major new initiative in the expansion of U.S. BNCT research beyond the current scope of the Brookhaven trials. A process developed by the INEEL for synthesis of decaborane, a key precursor for several advanced boron agents for BNCT, was licensed to a RADA partner, Neutron Therapies, Limited (NTL), and is now being synthesized for human use by NTL under FDA-approved Good Manufacturing Conditions. An Investigational New Drug Application (IND) that was submitted to the FDA for collaborative clinical trials of the first new boron agent synthesized via the new process was approved and human biodistribution studies related to treatment of both brain and lung tumors are anticipated to begin in early 1999. The parties to this collaborative effort are NTL, INEEL, and the University of Washington (UW) School of Medicine. Upon completion of the biodistribution studies, the cyclotron-based neutron radiotherapy facility at UW will be used, in conjunction with the new boron agent, for treatment of certain malignancies using BNCE-enhanced fast-neutron therapy. The INEEL has designed and tested a new neutron production target for this facility that will allow it to be more effectively used for this type of therapy as a result of the improved neutron spectrum that is produced by the new target. Future INEEL activities in connection with this collaborative effort will be focused on analytical chemistry for quantification of boron uptake in tissue, neutron beam dosimetry, and patient treatment planning. In a separate collaboration, construction was initiated on an advanced epithermal-neutron beam for BNCT research at Washington State University (WSU). This joint effort will provide a much-needed additional clinical-scale epithermal-neutron beam for BNCT research in the U.S. It will be installed in the thermal column of the existing TRIGA research reactor facility at WSU. Initial operation is scheduled for late 1999. INEEL contributions to this project have included the neutronic design of the beam, fabrication of key beamline components, assistance with installation, and in the future INEEL will perform experimental spectral characterization and dosimetry of the neutron beam that is produced. Continued participation by INEEL in advanced BNCT research at WSU is anticipated in the year 2000 and beyond.

(Supported by DOE OBER)

154. PPG Project III: New Analytical Methods in Kinetics and Tomography

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New analytical methods in kinetic modeling and reconstruction tomography of dynamic cardiac PET data will be investigated. Compensation for the compound motion (beating and respiratory) of the heart; model selection and parameter identifiability; and tomographic statistical efficiency are major areas of emphasis. Heart motion is the limiting factor determining quantification of tissue tracer concentration in cardiac PET. A detailed study of the bias intro-

duced in the estimation of cardiac tissue tracer concentration and the improvement attributable to new methods of compensation for heart motion is planned. Simulation studies and description length analyses are planned to investigate quantitative and qualitative differences among kinetic models with varying levels of physiologic detail. Particular attention will be given to the PET measurement environment and statistical properties of the measured data. Results will be used to select models for specific clinical objectives and to reconcile models used for kinetic cardiac PET and isolated perfused heart studies. Statistical efficiency of tomographic reconstruction algorithms and the statistically efficient estimation of functionals applied to volume tomographic datasets will be pursued with the objective of improved information extraction from existing PET technology and the reduction of patient dose and discomfort for PET instrumentation of the future.

(Supported by NIH/NHLBI, P01 grant)

155. Kinetic Modeling and SPECT

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With the advancement of multidetector SPECT systems, it has been demonstrated that dynamic cardiac SPECT imaging is possible with complete 360˚ angular sampling every 5 to 10 seconds. The development of dynamic cardiac SPECT has the potential to offer a more sensitive measure of myocardial ischemia than the present static SPECT techniques using Tl-201. Key to this development is the appropriate applied mathematical tools to accurately and precisely quantify kinetic parameters derived from dynamic cardiac SPECT data. The following tasks have been designed to develop the necessary tools: Task 1: Continue software development effort for estimation of kinetic parameters directly from SPECT projection data. Develop routines which model the acquisition of sinotimogram data which are unique to three-headed SPECT scanners. Incorporate attenuation, geometric response, and scatter thus far developed by the University of Utah. Evaluate feasibility of performing iterative nonlinear fitting of kinetic parameters routinely by networking to high performance computing facilities. Task 2: Assist the University of Utah with the determination of statistical uncertainties and correlations of region of interest data obtained from images reconstructed using maximum likelihood algorithms. Quantify advantages of incorporation of statistical uncertainties and correlations into the estimation of kinetic rate parameters from region of interest data obtained from images reconstructed using maximum likelihood algorithms. Task 3: Implement and test input function parameterization and fitting as part of the existing region of interest fitting package maintained at Lawrence Berkeley National Laboratory. Perform computer simulations to fully characterize the effects of input function parameterization. Compare these results with the noisy input function methods now in use. Investigate the possible improvement in precision and degradation of accuracy which should occur if smoothing constraints are applied to the input function parameters.

(Supported by Univ. of Utah NIH/NHLBI, R01 grant)

156. Flow and Metabolic Tracer Kinetics in Rabbit Heart

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The ultimate goal of these investigations is to provide positron emission tomography (PET) or single photon emission tomography (SPECT) with tracer methodologies that could give clinically useful information on myocardial perfusion, metabolism, and/or viability. The principal focus of this project is to use the isolated red blood cell–albumin perfused rabbit heart to evaluate specific flow and metabolic tracers in a well-controlled *in vitro* environment. The metabolic tracer that will be evaluated is ^{18}F-fluorodeoxyglucose (FDG). Two tracer kinetic models that have been used with FDG and positron emission tomography to quantify glucose metabolism will be investigated: the Patlak multiple-time graphical analysis and the Sokoloff compartment model. We

will test three hypotheses: 1) FDG non-equilibration will produce significant errors in the use of the Patlak graphical analysis to quantify myocardial glucose metabolic rate; 2) dephosphorylation of FDG-6-PO_4 during ischemia or perfusion with palmitate is a problem in the use of the Patlak graphical analysis; and 3) direct measurement of tracer delivery and distribution with the multiple indicator dilution technique will reduce the complexity of the Sokoloff compartment model. The flow tracers to be evaluated are radiolabeled rotenone compounds. The central hypothesis is that myocardial deposition of these mitochondrial-avid agents is principally determined by flow subject to modification by changes in mitochondrial function.

(Supported by NIH/NHLBI, R01 grant)

157. PPG Project V: Ionic and Metabolic Changes in Ischemia and Reperfusion

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During the past 30 years, three techniques have been developed that provide avenues for re-establishing blood flow to jeopardized potentially ischemic myocardium; 1) coronary artery bypass grafting (CABG); 2) angioplasty and other interventional techniques; 3) thrombolysis. Reflecting the clinical success in re-establishing perfusion, there has been a growing investigative interest in understanding the pathophysiology of ischemic reperfused myocardium. The overall goal of this project is to use the isolated isovolumic red blood cell/albumin perfused (RBC-perfused) rabbit heart and isolated buffer perfused rat heart to explore techniques that might provide clinically relevant information about the ionic and metabolic derangements resulting from ischemia and reperfusion. This project has been divided into two subprojects with two specific aims each. The first subproject will evaluate the fluorodeoxyglucose (FDG) methodology as a means of quantifying exogenous glucose utilization in the RBC-perfused rabbit heart. In Specific Aims 1 we will first evaluate the FDG approach at varying coronary blood flow rates using three tracer kinetic models: 1) Sokoloff-Phelps; 2) Patlak: and 3) impulse response. The multiple indicator dilution technique will be employed to study potential inaccuracies created by ignoring intracardiac tracer dispersion as currently practiced with PET and FDG. We will also extend our analysis of FDG methodology to the post-ischemic reperfused myocardium. Under Specific Aim 2, we will continue our evaluation of the use of FDG to quantitate glucose usage in low-flow ischemia and control-flow hypoxia. The direct testing of FDG tracer kinetic models will be done with perfused hearts in the PET scanner to be constructed in Project II. The analysis of data obtained in Project V will be performed in Core A and Project III. The perfused-heart methodology described in Project V will be utilized by Project IV for the analysis of radiolabeled flow and mitochondrial tracers.

(Supported by NIH/NHLBI, P01 grant)

158. PEREGRINE Program

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PEREGRINE provides accurate three-dimensional modeling of radiation dose for the treatment of cancer patients. The PEREGRINE dose calculation system merges Monte Carlo radiation transport methods developed in support of the U.S. nuclear weapons program with state-of-the-art computer technology to provide accurate dose calculations that are practical for everyday use in radiation therapy. This technology is of critical importance to the over 600,000 cancer patients who receive radiation therapy for cancer each year in the United States. The PEREGRINE group has also developed a method to incorporate these highly accurate calculations into an "intelligent system" that can customize the patient's individual treatment using computer-optimization methods. This will be the first time accurate Monte Carlo dose calculations and optimization techniques have been combined to do rapid and accurate treatment planning. Current results suggest that highly accurate dose calculations available from PEREGRINE will transform the field of radiation therapy in many ways: More cures (physicians will be able to accu-

rately target the patient's tumor), less complications (physicians will have reliable means to avoid sensitive structures), better clinical trials (multiple institutions will be able to accurately compare their results), and enabling new radiation delivery technologies (improved modeling capabilities will enable faster examination and implementation new radiation delivery devices).

(Supported by LLNL internal funding /NIH)

159. Modeling of Laser-Tissue Interaction

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Lawrence Livermore National Laboratory (LLNL) scientists are using computers to model the interaction of laser light with biological tissues. Modeling is used to design experiments and to gain a deeper understanding of specific laser-medical processes. In addition, it facilitates a more rapid development of instruments and ideas.

(Supported by Industry /LLNL internal funding)

160. TeleMed

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A "virtual" electronic record that represents a patient's medical record with pointers to actual data was initially developed by the Advanced Computing Laboratory of Los Alamos National Laboratory through internal discretionary research funding as an example of how complex multi-media data can be shared over the internet with security and data integrity. The research is now being funded by the US Army for sharing dermatology and dentistry consults over the internet. In addition, TeleMed is being deployed in northern New Mexico through a grant from the National Telecommunications Information Administration of the Department of Commerce. The objective of this project is to implement TeleMed as an electronic medical record-keeping system that will provide for both data management/ exchange and telecommunications within and between regional clinics and hospitals for immunization records. A Patient Identification Service (PIDS) will be used to identify the patient and the relevant immunization records wherever those data exist in the region of 16 rural clinics and two hospitals.

(Supported by US Army Medical Research & Materiel Command)

161. HIV Genetic Sequence Database (http://hiv-web.lanl.gov/) and HIV Immunology Database (http://hiv-web.lanl.gov/immunology/index.html)

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The HIV Genetic Sequence Database collects, organizes and analyzes HIV-1, HIV -2 and SIV genomic (DNA and RNA) and protein sequences. The overall goal is to track the global variability of HIV for purposes of aiding in vaccine design. Other benefits are basic research on lentiviral pathology and evolution, aid in design of diagnostic tests for HIV infection, and advancing the knowledge of evolution of virus-host relationships. The HIV Immunology Database collects, organizes and analyzes monoclonal and polyclonal antiboodies against HIV-1, HIV-2 and SIV. It also maps the location of antibody-binding epitopes on the HIV proteins. The goal is to aid vaccine developers and HIV researchers.

(Supported by National Institute of AIDS and Infectious Diseases, NIH)

162. Combining Diverse Uncertain Information in Bayesian Models: Applications to Functional Brain Imaging

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We have recently demonstrated a new probabilistic approach to the electromagnetic inverse problem for functional brain imaging based on Bayesian inference. Unlike other approaches to the problem, this approach does not result in a single "best" solution to the problem. Rather, a complete probability distribution of solutions is estimated (using Markov Chain Monte Carlo, upon which all subsequent inferences are based). In addition to emphasizing the inherent probabilistic character of the problem, Bayesian methods provide a formal, quantitative means of incorporating additional relevant information, independent of the measurements themselves, into the resulting probability distribution of inverse solutions. This approach has considerable generality and we believe it has value in both biological and non-biological applications well beyond the specific brain imaging applications studied to date. For example, we are actively exploring the value of the approach for Los Alamos global climate models.

(Supported by NIH, LANL, LDRD)

163. Modeling Influenza Outbreak

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Making use of a great deal of data that have been gathered on influenza, including sequence, serological and geographical morbidity and mortality data, scientists at Los Alamos National Laboratory and the Centers for Disease Control (CDC) are creating a model to predict the spread of an influenza pandemic worldwide. Using the next generation of supercomputers that will make it possible to obtain results in real time, it will be possible to predict how the spread of disease would be affected by closing certain air routes, for example. People most likely to be exposed to new strains of the flu first, such as customs officers, could be identified. More sophisticated versions of the model are anticipated that would include data from the CDC on the historic mutation of the influenza virus to help predict how serious a threat the emerging new mutation represents.

(Support from CDC pending)

164. LABEYE (Los Alamos Biomechanical EYE)

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LABEYE is a detailed biomechanical model of the human eye that was originally developed to provide a predictive capability for radial keratotomy. The model was successfully used in collaboration with Chiron Ophthalmics to assess the viability of a "smart" knife in RK surgery and to determine the causes behind the weak response to RK exhibited by patients having corneas that are significantly thinner (20% or more) than normal. In fact, the model is generally applicable to any type of keratorefractive surgery and could also be used to study changes in corneal tissue properties associated with karataconus and glaucoma. Only by using a model such as LABEYE can one hope to fully characterize the cornea [geometry, tissue properties, loading condition (IOP)] *in vivo*. Patent on non-contact intraocular pressure measurement sytem, which originated during work on the LABEYE project, is currently licensed by Interferometrics Inc. in Los Alamos, New Mexico.

(Support for LABEYE development was provided by Los Alamos National Laboratory internal research and development funds and Chiron Ophthalmics).

165. Virtual Safety Laboratory

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In order to provide a cost effective method for routinely evaluating the safety of new automobiles during the design phase, we are developing a Virtual Safety Laboratory (VSL) that complements and extends the knowledge base derived from full-scale crash testing. The VSL, which is to be validated on the basis of crash test data, provides a "point and click" graphical user interface that enables a designer to select a particular vehicle configuration, specify occupant anthropometry, import detailed biomechanical models for specified body components, select a particular type of collision, specify appropriate collision parameters, execute a test calculation, view animated results and assess the associated potential for occupant injury. The human occupant model developed at Los Alamos is based on a finite element model of the Hybrid III Anthropomorphic Test Dummy developed by the National Crash Analysis Center at Georgetown University under contract to the National Highway Traffic Safety Administration. To demonstrate the substitution capability of the VSL and provide a research tool for studying blunt body trauma to the head, we have developed a 60,000-node finite element model of the human head based on the Visible Human dataset provided by the National Library of Medicine. Many other biomechanical component models are being developed by other research organizations and could be incorporated into the VSL as they are validated and released.

(Originally supported by Los Alamos National Laboratory internal research and development funds and the General Motors Corporation. No current sponsor.)

166. Nanocrystalline Materials for Medical Applications

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Ti-^{6}Al-^{4}V alloy has been used to make medical implants for the past few decades. This alloy was initially developed for the aerospace industry. The medical industries later started to use this alloy for medical implants because of its high strength. However, there have been concerns about the toxicity of the alloy elements. They have the potential to cause cancer, inhibit apatite formation and cause neurological disorders. Recent experiments have shown that the implants made of the Ti-^{6}Al-^{4}V alloy do not fix to the bone tissue as well as the pure Ti implants. Pure titanium is chemically inert and compatible with human tissue, but large-grained pure titanium lacks the strength needed for implants. Recently, a new technology, Equal Channel Angular Pressing (ECAP), has been developed to process pure titanium into high-strength, non-toxic implants by refining the grain size. The technique can process coarse-grained pure Ti rod with a diameter of 20 mm or larger into an ultrafine-grained (grain size =200 nm) Ti rod with the same diameter. This ultrafine-grained Ti material has the potential to replace the current Ti alloy as the material for medical implants. Three more issues under investigation or to be investigated are i) scale up the Ti bar size up to 40 mm in diameter, which is necessary for large implants such as hip implants; ii) increase the fatigue property for applications where fatigue strength is a concern; and iii) biocompatibility tests. Although we expect the ultrafine-grained materials to be biologically compatible with the human body, some tests are desired to ensure the biocompatibility. Note that pure Ti has been approved by FDA for medical implants. Currently, LANL is collaborating with a Russian institute and two US companies to commercialize the ultrafine-grained Ti for making medical implants. One of the two US Companies, Intermedics Orthopedics, Inc., is one of the top three companies in the US making hip implants. It also has plans to use the ultrafine-grained Ti to make heart valves and pacemakers in its sister companies.

(Supported by DOE, Russian institute and two industrial partners)

167. Nonlinear Analysis of EEG for Condition Change

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Brain wave (EEG) data were examined from epileptic patients, under internal ORNL R&D sponsorship in 1994 and 1995. A zero-phase, quadratic filter was developed to remove the signals associated with low-frequency muscular activity, leaving the brain waves for detailed analysis. Nonlinear statistical measures of these artifact-filtered data clearly indicate an epileptic seizure. Related measures are predictive of the seizure 8 to 15 minutes prior to its clinical manifestation. The products of this work include two patents and one patent pending for epilepsy detection, epilepsy prediction (all claims allowed) (US Patent #5,743,860) and alertness monitoring (US Patent #5,626,145). Subsequent work in 1996 examined additional brain wave data under other internal ORNL R&D funding. A new approach was developed that captures the dynamical states in time-serial data as a phase-space (geometric) representation. The phase-space form subsequently is transformed into a probability density function (PDF). An assessment of condition change uses chi-squared statistics to measure the difference between a basecase PDF and a PDF for another time interval. The basecase PDF is typically for a non-seizure period. Other normal brain activity displays modest differences from the basecase, corresponding to the dynamics of thinking. Large differences indicate preseizure processes. The largest differences occur during an epileptic episode. US Patent #5,815,413 covers these improved indicators for epilepsy prediction and detection. This on-going effort is developing further improvements to the techniques. The goal is sensitive nonlinear metrics for determination of EMF effects in human EEG data.

(Supported by the DOE EMF Bioeffects Research Program and LDRD)

168. Therapy of Leukemia and Lymphoma with Monoclonal Antibodies

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The objective of this effort is to provide scientific and technical support to Fred Hutchinson Cancer Research Center, which involves calculations of medical internal radiation doses to patients scheduled to receive high-dose therapeutic administrations of iodine-131-labeled monoclonal antibodies for the treatment of acute leukemia and non-Hodgkin's lymphoma. Calculations are performed using biological retention data obtained from nuclear medicine imaging for specific organs and tumor tissues.

(Supported by Fred Hutchinson Cancer Research Center)

169. Internal Dosimetry for Antibody Development

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The objective of this project is to provide scientific and technical support to NeoRx Corporation's clinical studies involving the development and testing of new radio-pharmaceuticals and antibody targeting system for treatment of solid tumors in humans. This project involves calculation of retrospective medical internal radiation doses to patients after they have received therapeutic administrations of yttrium-90 and rhenium-186-labeled radiopharmaceuticals. Calculations are preformed using biological retention data obtained from nuclear medicine imaging for specific organs and tumor tissues.

(Supported by NeoRx)

170. Cancer Management Information System (CMIS)

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PNNL developed a prototype Cancer Management Information System [CMIS] for Madigan Army Medical Center in FY97. CMIS is an integrated information system for breast cancer demographics, prevention, detection and education for patients and health care providers. The core of the system is a kiosk designed as a multimedia tool to be used by the patient in the waiting room setting. This patient-oriented kiosk collects and stores as well as provides information. The patient information is provided in a format that allows the patient to review information and select more detail on subjects of interest. It also allows the user to access more detailed information to answer questions. PNNL is continuing to provide support to MAMC as it implements the system.

(Supported by Madigan Army Medical Center)

171. Bayesian Network for Confirming Diagnosis and Recommending Treatment Options

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The health care provider currently spends 1 to 2 hours per patient in determining possible tests to differentiate diagnoses, concluding with a diagnosis and determining the treatment plan. The time saved by using the Bayesian Network could be as much as 80% for both the physician and patient. This PNNL project involves constructing a Bayesian Network for diagnosis factors based on patient profiles. The current effort is focused on Obstructive Sleep Apnea Syndrome diagnosis and treatment options. Obstructive Sleep Apnea Syndrome would serve as the model diagnosis for further implementation of Bayes Nets to the Madigan computerized outpatient record.

(Supported by Army Artificial Intelligence Center in conjunction with Madigan Army Medical Center)

172. Assessing Depth of Anesthesia with Artificial Neural Networks

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The purpose of this project was to develop a method to assess depth and trend of anesthesia from electroencephalograms (EEG), upper facial electromyograms (EMG), and auditory evoked potentials during general anesthesia by using supervised artificial neural networks. The artificial neural network was trained to recognize the spectral differences in the signals between an awake and an anesthetized patient. A first study was completed with carotid endarterectomy patients.

(Supported by DOE ER CRADA with Cadwell Laboratory, Inc.)

173. Neurometric Assessment of the Effects of Analgesia

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Under a previous CRADA with Cadwell Laboratory, Inc., PNNL developed a software prototype that determines depth of anesthesia in patients during surgery. This software uses data collected with the Spectrum 32 instrument developed by Cadwell. PNNL is making minor changes to the software to analyze the EEG signal for the effects of analgesia. This project will also assess pain levels from the EEG signals.

(Supported by U.S. Army Institute for Surgical Research)

174. Information Analysis and Visualization Research and Development

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The purpose of this project is to extend the use of data visualization technologies to areas of interest to the U.S. Army Medical Research and Materiel Command. This project is follow-on work to an effort conducted for MRMC that evaluated the use of the PNNL developed SPIRE [Spatial Paradigm for Information Retrieval and Evaluation]Technology for use in "mining" information from the Army's extensive trauma databases. This project ranges from additional feasibility studies to signal processing research, language analysis research, and WebTheme development.

(Supported by Army Medical Research and Materiel Command)

175. The Rehabilitation Learning Center: A Network-Based Learning Environment for People with Disabilities

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The Rehabilitation Learning Center (RLC) is a Web-based learning environment that provides spinal cord injury patients with self-paced and customized rehabilitative training. Because of the Web-based nature of the system, patients may learn from within their own homes. The system is driven by the Pachelbel engine, which was developed at PNNL. Pachelbel is a Web-based, learning and information delivery system capable of tracking individual users' progress through a set of content. Pachelbel builds, reformats, and displays customized content based on specific user characteristics. Key characteristics might include the user's current position within the system, learning style preferences, contextual information, and the user's preferred mode of navigating through the material. The RLC project is the latest of several Pachelbel-based projects developed at PNNL.

(Supported by Center for Disease Control grant through Washington University School of Medicine)

176. Breast Cancer Prevention, Education, and Diagnosis Program

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The Pacific Northwest National Laboratory is assisting Keesler Medical Center (DoD Health Region 4) in the development of a Prototype Breast Cancer Education and Awareness Prototype Plan. The purpose of this project is to develop a comprehensive plan for patient identification, education, testing, and management of genetic predisposition to breast cancer with an ultimate intended application to all DoD health service providers.

(Supported by Keesler Air Force Medical Center)

177. Computational Molecular Recognition

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This is a new proposal to develop and apply software tools for computational molecular recognition. The software tools (docking, molecular dynamics, and quantum calculations) will be placed on a parallel framework that takes advantage of the large computing resources available to Sandia. With increased computer power, attention will be placed in the development of more realistic physical models for the molecular recognition calculations. The applications will be to find small molecules that specifically recognize toxins and could be useful as either sensor

materials or antitoxins. Particular attention will be placed on examining the toxins for conserved regions, and finding molecules that bind to those regions, so that they will be effective across multiple strains. The toxins targeted are tetanus, botulinum, staphylococcal enterotoxins (superantigens) and ricin. The tools for computational molecular recognition, taking advantage of computing resources at SNL, will be generally useful for the drug discovery process. They can be applied to search for novel lead drug compounds or improve existing ones. They can also be used to understand and model macromolecular interactions. The specific applications in tetanus, botulinum and superantigens (causing food poisoning and toxic shock syndrome) may lead to discovery of compounds that are promising lead drug compounds. Time frame: FY99 and ongoing.

(Supported by DOE NN/Chemical Biological Non Proliferation Program)

178. Molecular Theory of Gatekeeper Proteins

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The purpose of this work is to demonstrate that the current flow through ion channel proteins can be predicted from physical models at the molecular level. Ion channel proteins are important for a wide variety of biological function, including regulation of transport of ions into cells, signaling in the nervous system, and signaling for muscle contractions (including the heart muscle). Because of the importance of ion channel proteins in physiological response, a large fraction of drugs act on these proteins. The computational work to be performed in this project will be done with a massively parallel density functional theory (DFT) code (TRAMONTO) that was developed with LDRD funds (FY96–FY98). This is a new effort in biotechnology based on molecular modeling efforts that have been ongoing for a variety of other applications. Work will be conducted in collaboration with Bob Eisenberg (Rush Medical Center) and Lesser Blum (University of Puerto Rico). Understanding, and more importantly, being able to predict the properties of ion channel proteins will have enormous impact in both drug design and nanotechnology for bioengineering applications. Ion channels are one of nature's nanoscopic porous materials that exhibit a high degree of selectivity. They can pass a high current while maintaining their selectivity, and so are obvious candidates for sensors and separation membranes. Time frame: FY99 and ongoing

(Supported by Laboratory Directed Research & Development, DOE)

179. Information Integrity and Privacy for Computerized Medical Patient Records

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New health information systems offer the greatest potential in improving the nation's health and the quality of care its people receive. However, one of the major barriers to acceptance of these new systems is information surety: balancing confidentiality (or privacy), integrity, and availability of data. The main objective of the CRADA was to address privacy, integrity, and availability issues related to electronic health information and to develop methods that provided reasonable protection of electronic health information. Time frame: FY93–FY96.

[Supported by Oceania, Inc. (CRADA)]

180. Computational Biology and Molecular Recognition

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The development of computational methods for protein structure prediction, computational chemistry methods in molecular recognition, and genomic mapping was the focus of this project. Research in computational methods was devoted to Ab Initio

Lattice Protein Folding, Monte Carlo Simulations of Protein Misfolding, Structure Alignment/ Fold Classification, Computational Intractability of Protein Folding, Protein Threading and Design, Statistical Mechanics, Combinatorial chemistry methods in molecular recognition and drug design (Algorithm Design for Inverse Problems for Topological (2D) Chemical Indices and 3D Chemical Related to Lead Compound Optimization in the Design of Combinatorial Peptides and Combinatorial Libraries), and Combinatorial Methods for the STS Genomic Mapping with Repeated Probes. Powerful software methods for protein folding, molecular recognition and genomic mapping Tortilla Software Package were developed.

(Supported by DOE MICS Program, Laboratory Directed Research & Development, DOE DP)

181. Virtual Reality System for Training Emergency Medical First Responders

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BioSimMER is an existing prototype Virtual Reality system supporting training/rehearsal for NBC first responders. BioSimMER immerses the trainee in a high resolution synthetic environment consisting of a virtual environment, the participants, and virtual casualties. Each participant wears a VR head-mounted display and a set of position trackers that permit him to view and interact with the virtual world. The system also has a voice recognition capability. Multiple participants may share the simulation via a local area network (LAN) utilizing standard multicast protocols. Participants are represented within the VE as full graphical figures called Avatars. These Avatars provide a high fidelity representation of the actions and motions of the participant. The simulation itself consists of several components. Casualties are modeled using virtual humans that manifest the symptoms of the injuries being modeled as well as the changes brought about by the intervention of the trainee. The system provides feedback to the user on the state of the casualty and on the status of the procedure being performed. Initial funding for BioSimMER was provided by DARPA. Further funding has been provided by the Naval Health Research Center to extend the set of injuries from the initial tension pnuemothorax to head trauma and multiple injuries. Current DARPA funding supports extensions to multiple casualties and a WMD scenario (a biological agent release permitting modeling of agent exposure symptoms in addition to conventional injuries). This work addresses the need for experiential training focused on rapid situational assessment and decision-making under highly stressful conditions. The system augments traditional training (live exercises) in large-scale emergency medical response. Virtual Reality provides dynamic, hands-on simulations of large-scale disasters with casualties who both manifest the physiology of a given wound dynamically over time and who also respond to the medic's actions. In addition, it can present multiple scenarios, rare events, and alternate outcomes to provide a wide breadth of experience. Finally, it can realistically present the results of actions and decisions, both positive and negative, without the dire consequences of "learning under fire." Time frame: FY95–FY99.

[Supported by Defense Advanced Research Projects Agency (DARPA) and Naval Health Research Center]

182. Chemical Recognition Software

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The Development of a small portable sensor for optical detection of chemicals/biologicals for DoD applications, and in particular, intelligent software to distinguish signatures from monoclonal antibodies, and enzymes and their bound complexes. Sandia patent-pending software using multivariate genetic algorithms, and neural nets will make the determination. The detection of a single anthrax spore has been demonstrated. The single simple sensor should be capable of simultaneously detecting thousands of pathogens in real time. Time frame: FY96–FY99.

[Supported by Biopraxis Corporation (CRADA)]

183. The EGS (Electron Gamma Shower) Code System

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The EGS Code System simulates the radiation transport of electrons and photons in matter. It has been used extensively at SLAC to design high-energy physics experiments. There are about 6,000 EGS users worldwide. More than half are medical physicists and radiation oncologists, for many of whom EGS is one of the primary tools in radiotherapy and nuclear medicine for the treatment of cancer. To make EGS accessible to an even larger user base, SLAC plans to collaborate with Quantum Research, a company in Durham, NC, to develop a visual user interface for an enhanced EGS code with new and refined physics. The user can then easily build geometries and define Monte Carlo scoring routines, for example, in calculating radiation doses to tumors. For this work, Quantum Research anticipates an SBIR Phase II award in FY 99 to carry through a 2-year collaboration with SLAC.

(Supported by DOE SBIR)

184. Radiation Shielding for Hospital-Based Accelerators

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This project was done in collaboration with Varian Associates. SLAC created a computer model of the target assembly and shielding for a Varian 2100C medical linear accelerator. Monte Carlo computer codes were used to generate neutron source terms and to transport neutrons and photons from the target, collimator and field flattener assemblies, through the shielding. Neutron and photon intensity and energy spectrum distributions were calculated in a typical treatment room. Measurements were made at Varian to determine the transmission of neutrons and photons from a Clinac 2100C through concrete of varying density obtained from three different commercial suppliers. The following will be considered: Cost-effective shielding for medical accelerators (results will be incorporated into recommendations for shielding medical accelerator facilities and calculations should be made to extend the transmission measurement data). Medical applications include shielding of medical accelerator facilities and determination of radiation dose to patients outside of the treatment field. Medical categories include treatment and cost savings.

(Supported by NIH, National Center for Research Resources and DOE/OBER)

Biomedical Applications of Lasers

The various research programs encompass the development of new laser-based technologies and the application of these technologies in biology and medicine.

185. Optical Coherence Tomography for Periodontal Disease Detection

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Researchers at the Lawrence Livermore National Laboratory are developing an optical technique for non-invasive imaging of biological tissue. Optical Coherence Tomography (OCT) generates high-resolution (<20 micron) cross-sectional images of tissue without the need for tissue biopsy. The images are taken using near-infrared light, avoiding the dangers associated with ionizing radiation, as with x-ray images. Periodontal diseases are plaque-induced disorders that result in loss of connective tissue attachment and resorption of alveolar bone. An important aspect of periodontal disease assessment is determining the location of the soft tissue attachment to the tooth surface. Currently, mechanical or pressure sensitive probes are used to assess periodontal conditions. These probes can be painful for the patient and have several sources of error resulting from variations in insertion force, inflammatory status of tissue, and anatomical tooth contours. OCT is not sensitive to these errors and thus should be a more reproducible and reliable method for determining attachment level. Moreover, direct imaging of tooth and soft tissue structures *in vivo* may provide information that would allow diagnosis of periodontal diseases before attachment loss occurs.

(Supported by NIH / LLNL internal funding / Industry)

186. Ultra Short Pulse Laser Surgery

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The Ultra Short Pulse Laser (USPL) produces such short duration bursts of laser energy that surface material is removed without any significant transfer of energy to the surrounding areas. For laser pulses less than about 10 ps (1/100th of a billionth of a second), we've found that we can cut without collateral damage to surrounding tissues. By combining newly miniaturized commercial sources of ultra short pulse lasers with our delivery systems and diagnostics, we've created a powerful new surgical tool that creates tiny cuts with amazingly small kerf (>100 µm). We can also drill tiny holes (>150 µm diameter) all without thermal or mechanical damage to surrounding areas. Applications include optomological procedures, spinal surgery, dentistry and others.

(Supported by LLNL internal funding)

187. Tissue Welding

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Researchers at the Lawrence Livermore National Laboratory are exploring new means of joining tissue without sutures. Laser tissue welding uses laser energy to activate photothermal bonds and/or photochemical bonds. Laser tissue welding can be used either without sutures or staples or as and adjunct technique to improve suture or staple strength or

sealing characteristics. Tissue welding is a generic term that is also referred to as tissue fusion and vessel sealing. We use lasers because they provide the ability to accurately control the volume of tissue that is exposed to the activating energy. Currently, we are engaged in laser welding research for the repair of congenital aorta defects in neonates. The preliminary results show that blood leakage can be reduced by 70% when laser welding is used as an adjunct to conventional sutures in artery repair.

(Supported by Industry)

188. Laser Thrombolysis (clot removal)

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The Laser Thrombolysis Team is applying modeling and simulation expertise gained from nuclear weapons work to the task of perfecting a cost-effective, minimally-invasive laser catheter clinical procedure called laser thrombolysis. This method is used for removing blood clots in arteries and vein grafts without residual damage to the blood vessel wall. Through their modeling efforts, the LANL team members are making exceptional contributions to the scientific understanding of the physical phenomena underlying laser-induced clot removal and other medical procedures, such as laser retinal surgery and ultrasonic lithotripsy, which are dominated by bubble dynamics. Furthermore, they have extended their numerical simulations of laser thrombolysis to studies of instabilities in the radial collapse of spherical cavities, so their work is also producing greater understanding of weapons physics, inertial confinement fusion, and sonoluminescence

[Supported by DOE DP CRADA (with Oregon Medical Laser Center, Portland, OR, and Palomar Medical Technology, Beverly, MA)]

189. Laser Welding of Bone

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We have developed a fiber-delivered laser method of tack-welding fractured bone pieces together to potentially permit healing with proper registration and without the need for screws and plates. This is especially of potential value for reconstruction of maxilofacial bone structures following trauma (i.e., reconstructive facial plastic surgery) since small bone pieces could be held in place for healing, which would otherwise be removed. This method has not yet been tested with live animals.

(No current sponsor)

190. High Throughput Genomic Analysis

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The sequence information provided by the Human Genome Project presents enormous opportunities and challenges for biologists. As attention turns to issues of individual genetic variation and the function of gene products, there is a need for analyses that are both genome scale, enabling the parallel analysis of hundreds or thousands of genetic elements, and global scale, allowing the analysis of hundreds or thousands of individual samples. We have developed approaches to high throughput genomic analysis using microsphere-based flow cytometry. These approaches are based on our ability to sensitively and quantitatively analyze the hybridization and enzymatic modification of nucleic acids on microspheres. The hybridization and enzymatic reactions, which include ligation, polymerization, and cleavage, can be analyzed by flow cytometry in seconds without the need for a wash step. Simplified sample preparation in combination with a soluble solid phase (microspheres) makes these methods compatible with conventional laboratory automation instrumentation. The use of

commercially available soluble arrays of fluorescent microspheres allows multiplexed analysis of dozens or even hundreds of reactions simultaneously in a single sample. The assays developing have applications in the discovery and scoring of single nucleotide polymorphisms (SNPs), gene mapping, genotyping for diagnostics and therapeutics, gene expression analysis, bacterial strain detection and identification, and forensics. The flexibility, sensitivity, and throughput of microsphere-based flow cytometric approaches provide a versatile platform for genome and global-scale genomic analyses.

(Supported by DOE, OBER)

191. National Flow Cytometry Resource

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The National Flow Cytometry Resource, in its 17th year of continuous funding, is a Biotechnology Resource that has five components: technology research and development; collaborations; service projects; information dissemination activities; and training activities. Current research projects are development of DNA fragment size analysis, bead-based assays of molecular interactions, and rapid kinetic analyses of cellular processes. While, at the present time, all of these projects do not have direct medical applications, it is expected that in the future they will have a profound impact on medical care. Throughout the history of flow cytometry, numerous developments have found their way into clinical medicine. Immunophenotyping, the identification and analysis of immune cell subsets, is a widely applied technology in leukemia, lymphoma, and AIDS diagnoses. Although there are several courses that are focused on clinical applications of the technology, our research course draws about half of its international participation from the clinical cytometry community.

(Supported by NIH, National Center for Research Resources and DOE/OBER)

192. Single Molecule DNA Sequencing

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We are developing a technique for rapidly sequencing long, single strands of DNA. Our approach involves: fluorescent labeling of DNA with base-specific tags; anchoring a single, fluorescently labeled DNA fragment in the center of a flow chamber; digestion of the fluorescently labeled DNA with an exonuclease that sequentially cleaves the 3′ terminal nucleotide; and detection/identification (in order of cleavage) of individual, fluorescently tagged DNA bases by laser-induced fluorescence as they pass through a focused laser beam in an ultra-sensitive flow cytometer. This approach to DNA sequencing has the potential to sequence DNA at a rate of hundreds of bases per second. Even more important than the projected sequencing rate is the projected ability to sequence long fragments. We expect to attain read lengths of 10 to 30 Kb, thereby reducing greatly the need of sequencing overlapping regions characteristic of the conventional, electrophoresis based sequencing. In contrast to gel electrophoresis, our approach is not limited to 1000 bp fragments or by current cloning bottlenecks. Methods for rapid sequencing of individual DNA s will be an integral part of molecular medicine in the next century.

(Supported by DOE, OBER)

193. DNA Fragment Sizing by Flow Cytometry

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Genset is a genomics company that is focused on idientifying the genetic basis for drug response. They are interested in evaluating the applicability of our technology in support of their DNA sequencing effort. We are designing and constructing an instrument for delivery to Genset. In our flow cytometric approach to DNA fragment sizing, restriction fragments are stained with a fluorescent intercalating dye.

The dye binds stoichiometrically to the DNA so that the amount of dye bound is directly proportional to the fragment length. There is a large increase in the fluorescence quantum yield of the intercalating dye upon binding to the DNA making it unnecessary to remove unbound dye before analysis. The stained fragments are diluted to ~ 10^{-13} M and introduced into an ultrasensitive flow cytometer developed in our laboratory. The intensity of the fluorescence emitted by each individual fragment as it passes through the laser beam is a measure of the fragment size. Picogram samples are analyzed in less than ten minutes. This represents an increase in sensitivity of ~ one million and a reduction in analysis time of ~ one thousand over conventional electrophoresis. Fragments ranging in size from 212 bp to 440 kbp have been sized by this technique. The resolution exceeds that of PFGE for fragments larger than 10 kpb. In addition to high sensitivity and speed, our approach is quantitative—individual fragments are counted. The analysis is conformation independent—linerization of circular and supercoiled DNA before analysis is not required. Applications under development include (1) bacteria fingerprinting for species and strain identification in support of our effort in the detection and identification of hazardous bacteria (DOE NN20), and (2) PAC (P1-derived artificial chromosomes) and BAC (bacteria artificial chromosomes) clone characterization for the construction of DNA libraries in support of large DNA sequencing projects.

(Supported by DOE (NN20), NIH funded National Flow Resource Federal Bureau of Investigation. Genset, a private company located in France with a large effort in San Diego, is partially supporting this work with a 100% funds-in CRADA.)

194. Laser Desorption Mass Spectrometry for Disease Diagnosis and Forensic Applications

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Rapid DNA sequencing analysis allows a range of biomedical applications to be considered, especially in the detection and diagnosis of genetic diseases. During the past 7 years, we have been developing laser desorption mass spectrometry (LDMS) for rapid DNA analysis and sequencing for the Human Genome Project. Current DNA analysis mostly relies on gel electrophoresis, which is a time-consuming process and requires radioactive material or dye tagging for detection. Furthermore, DNA segments with a high GC ratio, a high number of tandem repeats and/or secondary structures are often extremely difficult to characterize. With LDMS, all the above disadvantages can be eliminated. To date, we have succeeded in sequencing ss-DNA up to 125 nt and ds-DNA up to 250 bp using Sanger's approach to produce DNA ladders. To our knowledge, this represents a record for sequencing with by mass spectrometry. We have also succeeded in sequencing DNAs with the Maxam and Gilbert chemical degradation method to produce DNA ladders. With this approach, we can sequence DNA segments with a high G component and/or secondary structures since the band compression observed in gels is no longer a concern. We have also developed an innovative direct sequencing method to achieve rapid sequencing without the need of DNA ladder preparations. Thus, the overall sequencing time can be reduced to less than one second. We are among the first to apply LDMS for rapid disease diagnosis. By collaborating with Dr. Matteson at The University of Tennessee Medical Center, we have succeeded in investigating more than 30 clinical samples for cystic fibrosis (CF) by observing the F508 base deletion and G551 point mutation. We also work with Dr. Potter on neuro-degenerative diseases due to dynamic mutations such as Huntington Disease (HD) and dentatorubral-pallidoluysian atrophy (DRPLA). This work has the implication that LDMS can become a major tool for population screening for various genetic diseases. More recently, we also applied LDMS for DNA fingerprinting for forensic applications. The preliminary results indicate LDMS can be efficiently used for short tandem repeat (STR) analysis for the identification of individuals. In conclusion, LDMS is emerging as a new biotechnology for DNA analysis. It is valuable not only for the present Human Genome Project but also for gene function study for the post-genomic era.

(Supported by DOE OBER, National Institute of Justice, NIH)

195. Laser-Induced Fluorescence for Cancer Diagnosis

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In-situ and rapid procedures for tissue diagnosis are important for efficient cancer prevention and therapy. For instance, in humans, endoscopy is used to detect abnormal tissues in the esophagus. Differentiation of normal tissues and early adenocarcinoma is often difficult with current technologies Once an abnormality is found, biopsies are taken for determination of histopathology. The laboratory results are generally not available for several days. We have developed a unique "optical biopsy" technique using laser-induced fluorescence (LIF) that can provide effective indices to diagnose malignant tumors in the esophagus. The method was successfully tested with over 100 patients in collaboration with The Thompson Cancer Survival Center. The proposed project involves further development of the second-generation technology of LIF optical biopsy based on a novel SL detection methodology for monitoring small changes in fluorescence profiles of normal and tumor tissues. The novel approach is currently US Patent pending. The conventional laser-induced fluorescence (LIF) technique does not often provide the spectral specificity needed to provide clear "spectral fingerprints" of normal and tumor tissues. One methodology that has the potential of improving the LIF technique is the wavelength synchronous scanning method, often referred to as synchronous luminescence (SL). This novel SL detection scheme could lead to significant advances in effective detection of tumors and in the understanding of cancer therapy in general and a decrease in costs for cancer diagnostic and therapy.

(Supported by OBER, CRADA)

196. Semiconductor Materials Science Enables a Biological Microcavity Laser for Early Detection of Disease

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Based on a physical understanding of artificially-structured semiconductors, we have synthesized quantum well semiconductor heterostructures that efficiently generate intense light in a microscopic cavity and enable novel microfluidic sensing methods for environmental monitoring, biological detection, and early diagnosis of disease. One embodiment of this work is the biological microcavity laser which is a revolutionary method for analyzing biological cells. In this device, a single human cell acts as an integrated component of a semiconductor laser. The cell actually aids the light-generating process, so that the emitted laser beam is impressed with information about the cell. The technique does not require the customary chemical staining procedure to render the cell structure visible. This new laser provides the basis for new biomedical analyses of cell structure which can distinguish between healthy and cancerous cells. The biocavity laser is well suited to identify and rapidly assess the condition of biological cells and with further development could enable a novel hand-held tool for resecting tumor margins in surgical procedures. The tool would aspirate tissue, filter, separate, and collect cells in a form of on-line cytometry. Using microlaser spectroscopy in an inexpensive, small, ultrafast, microfabricated flow device, cells would be analyzed (in their physiologic condition without the use of staining) in real time as they are removed from the tumor. Information is impressed onto a circular laser beam ideally suited for coupling into a high-speed fiber-optic link for analysis in a high-speed computer, results instantly feedback to surgeon. The surgeon can characterize tumor cells to locate tumor margins and minimize trauma (e.g. prevent loss of vital brain function in neurosurgery). Time frame: FY97 and ongoing.

(Supported by BES Materials Science Program, Laboratory Directed Research & Development)

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Therapy

This section describes research projects involving radionuclide therapy, needle guided proton therapy, and chemotherapeutic drug delivery for effective cancer treatment. The projects also include catheterization research for delivering therapeutic amounts of energy to disrupt thrombus occlusion in stroke and prevention of restenosis, and encompass a portable device for separation of oxygen from air for emergency medical use at remote settings.

197. IMP Dehydrogenase as a Target Enzyme to Screen Novel Antimicrobial and Immunosuppressive Drugs

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This research focuses on IMP dehydrogenase (IMPDH), a target enzyme for novel immunosuppressive, anticancer, and antimicrobial drugs. IMPDH is critical in the nucleotide biosynthesis of guanine and, consequently, crucial for RNA and DNA production. Argonne scientists have cloned and characterized the mammalian forms of the enzyme. Other studies have shown that IMPDH is elevated in human tumors and that inhibitors of this enzyme restrain the replication of various types of human cells—lymphocytes in particular—and evoke terminal differentiation in human tumor cells. Because of these studies, IMPDH inhibitors are being developed as immunosuppressive and anticancer drugs. IMPDH inhibitors also have potential utility as antimicrobial agents because mammalian enzymes, including the human enzyme, differ from the bacterial IMPDH with respect to kinetics parameters and responses to certain IMPDH inhibitors. For this reason, Argonne has developed a screening method to identify new IMPDH inhibitors that may represent novel antimicrobial drugs. In addition, crystal structure determinations and site-specific mutagenesis studies will provide the Laboratory with rational bases on which to design such drugs. Regarding intellectual property, one patent was awarded and one application is pending.

(Supported by DOE, Office of Nonproliferation and National Security, NN 20)

198. DNA Repair Enzyme–Liposomes: Human Skin Cancer Prevention

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This project has a principal goal of developing and testing the efficacy of liposomes that contain DNA repair enzymes in removing sunlight-induced DNA damage from mammalian cells. Ultraviolet radiation (UV) is a potent carcinogen, inducing damages in the DNA of exposed cells. There are several cellular mechanisms for repairing DNA damage, mediated by at least three separate enzymatic pathways. In the absence of repair, malignant skin cancers develop at high frequencies. DNA damage repair by patients lacking repair enzymes can be effected by delivery of exogenous enzymes in liposome vehicles. This project evaluated the effectiveness of such liposomes containing repair enzymes to remove DNA damages and to lessen the detrimental effects of UV radiation.

(Supported by DOE OS/LTR Partner—Applied Genetics, Inc.)

199. Investigation to Assess the Influence of Topical Treatment with a Hydroxy Acid on UV β-Induced Thymine Dimers in Human Skin

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This project will assess the DNA damaging effects of certain cosmetic agents applied topically to skin. The FDA has become concerned that a cosmetic preparation (marketed as anti-wrinkle cream) containing α-Hydroxy Acid, which is being used widely and with increasing frequency, is reported to induce sun-sensitivity (sunburning) in individuals who were not previously sun-sensitive. Because increased erythemic responses of this nature are usually associated with DNA damage and because specific sunlight-induced DNA damage (cyclobutyl pyrimidine dimers) has been shown to be a molecular alteration leading to sunlight-induced skin cancers, this may indicate that the preparation increases DNA damage in treated and exposed skin. This research project shall ascertain whether this preparation does increase DNA damage in human skin exposed to ultraviolet radiation.

(Supported by FDA via KGL, Inc.)

200. DNA Lesion Clusters in Space Radiation Damage

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This project has an ultimate goal of minimizing radiation risks to space travelers by evaluating lesions in DNA induced by space radiation (protons and HZE particles) and determining methods to reduce the levels of such damages. The project has three phases: (1) measure the frequency of induction of specific clustered lesions in DNA in mammalian cells by space radiation; (2) determine whether the spectrum of clustered lesions produced by protons and HZE particles can be altered by chemical or genetic means, and measure the resulting levels of each cluster type; and (3) measure the relative rates of repair/rejoining of lesion clusters, compared with frank double strand breaks and isolated lesions of the same types in normal, repair-enhanced and repair-deficient mammalian cells, and determine whether changing the cluster spectrum affects repair, survival or mutation induction.

(Supported by NASA)

201. Effect of Imiquimod on UV-Induced Damage in Human Skin

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This work will assess the DNA damaging effects of agents applied topically to skin. Several such agents, with cosmetic or medical uses, may increase sensitivity to ultraviolet-induced erythema (sunburn). This study will determine if the increased erythemic response is accompanied by increased DNA damage (as in normal skin responses to sunlight) or whether the skin responses are independent of DNA damage. The occurrence of increased skin damage would be of great concern, as the specific type of DNA damage that we measure (cyclobutyl pyrimidine dimers) has been shown to be a molecular alteration leading to sunlight-induced skin cancer. One of the preparations to be tested is Imiquimod (5% cream), which is effective against genital warts, making it's known association with increased erythema of little concern because of the area of application. However, because the manufactures are proposing to use this preparation to alleviate actinic keratoses and other skin lesions on skin areas normally exposed to sunlight, it is critical to ascertain whether this preparation increases DNA damage in human skin exposed to ultraviolet radiation.

(Supported by KGL, Inc.)

202. Recombinant Vehicles for Tumor Imaging and for Targeted Radioisotopic/Genetic Therapy of Cancer

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The objective of this project is to develop and evaluate a number of recombinant vehicles, including bifunctional fusion proteins with and without adenoviral vectors, for the selective delivery of radionuclides and therapeutic and/or reporter genes onto tumor cells for imaging tumors, gene expression and for therapy. Adenoviral "vehicles" will be retargeted by two different approaches. (1) Using recombinant DNA techniques, functional domains of an adenovirus protein known to mediate virus internalization and virus entry into the cell cytosol will be fused to sFv minibodies against well-characterized tumor-antigens. (2) Adenovirus vectors carrying reporter genes will be retargeted to bind and infect tumor cells by incorporating tumor antigen-specific sFv's onto the virus capsid. Such retargeted adenoviral vectors and vehicles can be used for tumor imaging, radioimmunotherapies and for delivery of genes in a gene therapy approach.

(Supported by DOE OBER)

203. Microbeam Radiation Therapy for Pediatric Brain Tumors

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At Brookhaven National Laboratory we have pioneered a new method of radiation therapy, microbeam radiation therapy (MRT). MRT consists of arrays of parallel microplanar x-ray beams (MBs) generated by a synchrotron beamline. Typically, the beams were 30-μm wide and up to several centimeters high and were spaced 100 μm apart, center-to-center, with a half-spectral-power energy of 50 to 70 keV. MRT has two significant, remarkable effects. First, there is a sparing effect in normal tissue. When the rat CNS was irradiated in a single fraction with arrays of MB, neurons did not undergo necrosis after in-slice skin-entrance doses up to ~400 Gy, nor did brain-tissue necrosis developed after doses up to ~5,000 Gy (i.e. after doses that exceeded by ~10- to 100-fold the threshold for severe radiation damage from a wide beam). Second, unidirectional MRT has a preferential tumor-killing effect which has been observed in rats bearing a malignant tumor in their brain, or under their skin. Our postulates are that a) normal tissue endothelial and oligodendroglial cells regenerate from their precursors surviving between individual microbeams and that b) the inefficiency of this regeneration in brain tumors is therapeutic. Many years of preclinical research and technical development is necessary before the possible start of a clinical trial. Our goal for the first such trial would be for pediatric brain tumors, as the existing radiation therapy method often leaves neurological deficits in these children because of the extraordinary sensitivity of the developing brain to ionizing radiation

(Supported by DOE OBER)

204. Auger Electron Therapy: Gadolinium & Thermal Neutrons

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Boron neutron capture therapy (BNCT) and photon activation therapy (PAT) are two radiotherapeutic modalities in which stable target atoms attached to cancer seeking compounds are activated by a beam of thermal neutrons for the former and by photons for the latter. The result with both is the delivery of a dose of radiation to the tumor which exceeds that to normal tissues. The radiation delivered to the tumor is high LET by virtue of heavy particles in BNCT and Auger electrons in PAT. Of key importance is that Auger electron emission can be stimulated by thermal neutron capture, with resultant internal conversion of orbital electrons. Copious Auger cascades can be stimulated by this process, particularly if a

target atom such as gadolinium-157, which has a unique thermal neutron cross section of 255,000 barns, is used. ^{157}Gd has been attached to a tumor seeking porphyrin which also is tagged with boron. This development holds forth the promise of being able to apply, to a single tumor, the substantial advantages of both BNCT and Auger Electron Therapy (AET). The objective of this proposal is to exploit this potential, particularly in the medical management of patients with malignant brain tumors such as glioblastoma multiform. To evaluate the overall approach, ^{157}Gd attached to the porphyrin will be tested and compared with ^{10}B attached to the porphyrin. Comparison will then be made using the porphyrin with both ^{157}Gd and ^{10}B attached to the same molecule. To evaluate AET with Gd and thermal neutrons, a) in vitro survival assays will be used to compare ^{10}B and ^{157}Gd as target atoms, b) Monte Carlo electron track structure codes will be used to confirm the local nature of the biological effects of ^{10}B and ^{157}Gd, and to predict dose, c) in vivo drug distribution studies will be used to optimize delivery of ^{10}B and ^{157}Gd to tumor, d) preliminary toxicity studies will be undertaken to evaluate the tolerance of the drug regimen established in (c), and e) AET and BNCT will be compared in therapy studies using both mice and rats.

(Supported by NIH)

205. Neutron Capture Therapy: Preclinical Research and Clinical Investigations

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The long term goal of this project is to develop boron neutron capture therapy (BNCT) into a clinically useful radiation therapy. The initial clinical studies will focus on glioblastoma multiforme. The boron delivery agent will be the amino acid analog *p*-boronophenylalanine (BPA). Radiobiological studies with rats bearing intracerebral and/or subcutaneous gliosarcomas, with rat spinal cord, and with dog brain have quantified the biological efficacy of BPA-based BNCT relative to conventional photon radiation. Effective tumor control with little or no damage to adjacent normal tissue has been demonstrated. Clinical BNCT irradiations of glioblastoma multiforme are under way under an FDA-sanctioned Phase I/II trial. The trial is in the dose escalation phase. Animal studies will continue in concert with clinical application of BNCT so as to address specific issues arising from the clinical trials and to examine the utility of fractionated BNCT.

(Supported by DOE OBER)

206. Molecular Biological Markers as Potential Prognostic Indicators for BNCT

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In the patients treated with BNCT, palliation of the tumor was achieved in all cases, but the time to progression could not be correlated with clinical prognostic indicators. In this project we search for genetic and molecular markers that could be of use in predicting the course of the disease and to correlate their presence in actual patient tumor samples with clinical outcome. The aim of this work is to evaluate the biological markers from samples obtained from BNCT patients and to study the correlation of these markers with the patient survival following BNCT. Markers shown to have significant correlation with patient survival could then be used prospectively to identify candidates for BNCT. Cytogenetic and molecular genetic studies of malignant gliomas have documented abnormalities in many autosomes especially the loss of heterozygosity for loci of chromosomes 10 and 17 (p53) and amplification of EGFR gene (chromosome 7). The amplification of the gene for EGFR protein leads to its being over expressed in most malignant gliomas, especially in glioblastoma multiforme (GBM). Patients expressing p53 protein (product of the tumor suppressor gene on chromosome 17) have significantly reduced survival. Both EGFR gene product and p53 protein can be evaluated on formalin-fixed, paraffin-embedded sections obtained from BNCT patients. Apoptosis (active cell suicide controlled by gene expression), combined with proliferative potential of tumor cells has also been proposed as a

possible prognostic indicator. Apoptotic cells in the available formalin-fixed, paraffin-embedded tumor samples can be identified by the detection of DNA breaks by terminal transferase-mediated in situ end labeling (TUNEL). The combined outcome of this study will provide a more complete diagnostic profile that will serve two purposes: (1) it will identify patients that will most benefit from BNCT and (2) it will help to clarify our understanding of the underlying mechanisms leading to the development of glioblastoma multiforme. Such understanding may eventually lead to methods of preventing this devastating cancer.

(Supported by LDRD)

207. Biodistribution, Toxicity & Boron Neutron-Capture Therapy in Animals Using a Metallotetra–Carboranyl–Porphyrin Imageable by SPECT

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A tetracarboranyltetraphenylporphyrin [TCP], a chelator of nickel, copper or manganese, has been synthesized as a boron carrier for boron neutron-capture therapy [BNCT]. Animal studies demonstrated improved biodistribution using the TCP carrier as well as reduced toxicity. Little or no behavioral, chemical or hematological toxicity was observed up to 3 months after the injections. We shall carry out biodistribution and toxicity experiments in rats bearing subcutaneous gliosarcomas to optimize the administration method and the dosing schedule for maximal tumor uptake, minimal blood and brain uptake with tolerable or no toxicity. Analogues of TCP will be synthesized and tested to establish a BNCT-relevant structure–function relationship within this class of porphyrins. SPECT-imageable and MRI-imageable TCP chelates can be prepared and tested as tracers for the boron carrier to optimize the timing of neutron irradiation for each patient.

(Supported by LDRD)

208. Microdistribution Studies of Boron-10

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In radiotherapy the dose that can be delivered to tumor cells is limited by the tolerance of the normal tissues within the radiation field. Boron neutron capture therapy (BNCT) is based on the neutron capture reaction $^{10}B(n,\alpha)^{7}Li$. The alpha (α) particles have an average linear energy transfer (LET) of 196 keV/μm and a range in soft tissue of 9 μm while the lithium-7 particles have an average LET of 162 keV/μm and a range of 5 μm. The combination of high LET and a short range, approximately the diameter of a single cell, increases the statistical chances that ^{10}B-laden tumor cells will be rendered nonclonogenic ("killed") while contiguous, ^{10}B-poor tissues will be spared. BNCT involves preferential accumulation of a compound containing ^{10}B in the tumor and slow neutron irradiation of the target volume. Because of the diffusion of slow neutrons, tissues surrounding the target volume are inevitably irradiated. The microdistribution of ^{10}B in both tumor and normal tissues is of critical importance to BNCT. Information regarding the localization and concentration of ^{10}B in individual tumor cells, especially in tumor cells infiltrating beyond the main tumor mass, is essential in the understanding of the outcome of the current clinical trial and in the design of the future protocols. Many analytical techniques have been used to study the microdistribution of boron. Among them, secondary ion mass spectrometry (SIMS) has the best sensitivity and spatial resolution but is the least used. At present, only the SIMS group in the Butler Laboratory at Cornell University, Chemistry Department, has the expertise and equipment to perform SIMS-based ^{10}B analysis of cryostat tissue sections. Results from SIMS are of high quality but too sparse to fully support BNCT research at BNL. We propose to conduct a feasibility study on the ppm intracellular boron detection using the two high-performance transmission electron microscopes (TEM) at BNL. The goal is to measure the ^{10}B concentration in single cells and contiguous zones of extracellular matrix (blood boron analysis will be performed separately). These techniques will

provide much-needed information for BNCT clinical trials as well as for boron drug development at BNL.

(Supported by LDRD)

209. Brookhaven Medical Research Reactor Operations

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The Brookhaven Medical Research Reactor (BMRR) is a 3-MW reactor constructed primarily for medical and biological research. This proposal will provide the funding required for the operation and maintenance of the reactor facility in support of clinical trials of Boron Neutron Capture Therapy (BNCT). Although the BMRR has the capacity for isotope production and activation analysis, its two primary experimental facilities are designed to provide intense neutron beams for BNCT research. A clinical trial under way since FY 95 includes BNCT treatment of patients with glioblastoma multiforme. Upgrades to the facilities have been designed to reduce treatment times and to enhance the quality of the treatment by improving the beam penetration through tissue and to reduce unwanted effects from fast neutron and gamma contamination of the epithermal neutron beam.

(Supported by DOE OBER)

210. Phase I/II Therapy with Tin-117m DTPA

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The main focus of this CRADA project is for BNL/Diatide to conduct and complete research to optimize and test the modified tin-117m DTPA formulation for treatment of primary and metastatic cancer in bone. Objectives of this research project include the development and testing of the application of modified tin-117m stannic DTPA (molar ratio of DTPA to tin = 3) for the treatment in humans of primary osteoblastic osteosarcoma and bone metastases originating from this and other primary malignancies. If the results are promising, Diatide will pursue this project further and attempt to commercialize the technology. Each year approximately 400,000 cancer patients in the U.S. alone develop bone metastases. A majority of these patients (>75%) experience severe, chronic bone pain that results in significant or total immobility and a deterioration in the quality of life to a very poor condition. Since life expectancy can be rather long, even in patients with advanced metastatic bone disease, control of pain eventually becomes a major factor in clinical management. Relief of pain can be achieved with hormonal therapy for a period of time, but eventually symptoms become refractory. Chemotherapy has achieved limited success. Use of strong opiates has adverse consequences such as dulling of sensorium, constipation, etc. Radiation therapy is effective for focal areas but becomes difficult when the disease is widespread. Compared to these conventional treatments, certain radiopharmaceuticals labeled primarily with electron emitters have shown promise for providing safe, rapid and more effective relief of metastatic bone pain. A formulation of modified tin-117m DTPA has proven to be an effective palliative treatment for bone pain. This study will expand the application of modified tin-117m DTPA to treatment of primary and metastatic bone cancers.

(Supported by DOE SS/LTR Partner—Diatide, Inc.)

211. Vaccine Intervention for Lyme Borreliosis

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Proteins normally found on the surface of the Lyme disease spirochete, *Borrelia burgdorferi*, affect the antigenicity, immunological reactivity and host cell interaction of the spirochete. These proteins are the major focus of current efforts to develop safe, effective vaccines to prevent Lyme disease. The overall efficacy of a recombinant vaccine will depend upon the selection of one or more immunoprotective

target(s). Genetic variation, particularly the heterogeneity of surface proteins, can alter the antigenicity of the immunoprotective epitopes of the target proteins. Rational development of effective vaccines, therefore, requires determination of the sequence variation of the major outer surface proteins in *B. burgdorferi* and integration of this knowledge with the tertiary structure of these proteins to help define those regions where changes will influence interaction with antibodies. In order to provide a starting point to elucidate the molecular details of the interactions of the Borrelia surface proteins with the immune system we are attempting to use crystallographic techniques to solve the structures of Borrelia's major outer surface proteins alone and in complexes with Fab fragments of protective monoclonal antibodies. Experiments are also described aimed at development of multipeptide chimers to expand the immunoprotective potential of a given vaccine candidate. In particular, we shall determine the location of protective epitopes within the outer surface proteins and then use these regions to construct multipeptide recombinant hybrids that would extend immunization simultaneously to more than one genospecies and therefore have broad applications in prevention and diagnosis of Lyme disease.

[Supported by State University of NY, Stony Brook (NIH)]

212. Retargeting of Adenovirus for In Vivo Gene Therapy

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In collaboration with Dr. V. A. Svyatchenko and his staff, we will construct adenovirus mutants that will serve as the backbone for a new generation of retargeted virus vectors for *in vivo* gene therapy. Specifically, we propose to ablate the natural tropism of human adenovirus serotype 5 (Ad5) to serve as the starting material for preparation of a retargeted genetic vector. These mutants will allow the production of modified virus vectors that are unable to bind to the natural host cells for Ad5, thus maximizing delivery of the Ad5 vectors to novel cellular targets. Gene therapy is defined as the transduction of cells with genes that can either directly correct genetic defects or transform cells in ways that augment other forms of therapy. For example, the introduction of normal or recombinant genes to produce functional proteins would correct diseases resulting from recessive genetic disorders such as cystic fibrosis. Introduction of the multidrug resistance gene into bone marrow stem cells would render the progeny of these cells more resistant to drugs used in chemotherapy, which would relieve the myeloablation normally resulting from chemotherapeutic regimens used in cancer treatment. The results of this work will be applicable to all current and future genetic therapies.

[Supported by DOE; Albuq. Op. Office; Participating Group: State Research Center of Virology and Biotechnology (Vector), Koltsovo, Russia]

213. Regulation of Adenovirus Proteinase by a Peptide J DNA

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Among potential targets for antiviral therapy that arise during certain viral infections are the virus-coded proteinases. These enzymes, essential for the synthesis of infectious virus, are required to process virus-specific precursor proteins involved in the maturation, assembly and replication of such pathogenic human viruses as adenovirus, poliovirus, encephalitis virus, hepatitis A and C viruses, cytomegalovirus, and human immunodeficiency virus. Virus-coded proteinases are highly specific for their virus-coded substrates. If equally specific inhibitors can be developed and targeted to infected cells, they should interfere with virus replication and not with normal cellular metabolism. Our model system is the infection of HeLa cells in culture by human adenovirus serotype 2 (Ad2). We showed for maximal Ad2 proteinase activity in vitro, three components are required—the protein product of the adenovirus L3 23K gene, an 11 amino acid peptide (pVlc) that originates from the C-terminus of virion precursor protein pvI, and the viral DNA. The cofactors increase k(cat), 300-fold with pVlc and 6000-fold with Ad2 DNA as well. We solved the three-dimensional structure of the proteinase complexed with pVlc at 2.6 Angstrom resolution. The fold of the protein is

unique. A putative active site contains a Cys–His–Glu triplet and oxyanion hole in an arrangement similar to that in papain. If that is the active site, this protein would represent a new class of cysteine proteinases and a new, fifth group of enzymes that contain catalytic triads. Here we propose to understand at the biochemical and structural levels how the activity of the adenovirus proteinase is regulated. We shall locate and characterize the active site, elucidate the mechanisms by which the two cofactors stimulate proteinase activity and characterize the enzyme during different times after infection. We have just shown that beta-actin can serve as a cofactor and will characterize that interaction with the enzyme in vitro and in vivo. Although important in themselves, the results of these experiments will also be used in a subsequent grant to design different types of proteinase inhibitors to act as antiviral agents.

(Supported by NIH)

214. Radiobiological Effects of Electrical Stimulation on Normal and Malignant Human Cell Lines In Vitro

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The Neutron Therapy Facility (NTF) is currently collaborating in a clinical trial that uses electrical stimulation to reverse side effects of radiation therapy, including progressive development of scar tissue, pain, decreased range of motion, etc. in patients who have completed courses of radiation therapy. Preliminary results indicate that the therapy is effective for side effects that develop many months after treatment. There is anecdotal evidence that the effects may be prevented or significantly reduced if the electrical therapy is given concurrently with the radiation therapy. Before entering into such a clinical trial it is appropriate to measure the effect of the electrical stimulation on malignant cells to be sure that the stimulation does not "heal" tumor cells damaged by radiation. In collaboration with Rush University, NTF has begun radiobiological studies of prostate cancer cells combining electrical and radiation therapy. We also intend to study brain tumor cells and healthy epithelial cells. This study has direct bearing on quality of life issues for all patients receiving any form of radiation therapy. There is a potential for making dramatic improvements in quality of life for these individuals.

(Supported by internal funding)

215. Radiobiology of Bromodeoxyuridine as a Radiation Sensitizer in Fast Neutron Therapy for Brain Tumors

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Clinical trials with conventional therapy show an increase in survival time for advanced brain tumor patients who received bromodeoxyuridine BrdU concurrently with radiation therapy. In vitro studies done at Brookhaven National Laboratory found an even greater tumor killing affect for brain tumor cells treated in a neutron beam. Once the vertical computerized axial tomagraphy (CT) scanner is commissioned the Neutron Therapy Facility (NTF) physicians intend to start a clinical trial using BrdU to sensitize brain tumors to fast neutrons. Hence, we are beginning in vitro studies to estimate the sensitization effect of BrdU as a function of its concentration in the tumor. This study has direct bearing on treatments designed to prolong the lives of people suffering with brain tumors.

(Supported by internal funding)

216. Boron Neutron Capture Enhanced Fast Neutron Therapy (BNCEFN)

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The Neutron Therapy Facility (NTF) is collaborating with physicians and physicists at Orsay, France in modifying clinical fast neutron beam spectra to

augment the low-energy component, thus increasing the effectiveness of the dose to a tumor containing boron-10. We have succeeded in producing and measuring an augmented low-energy component. We are now designing experiments to test collimation schemes. At the most recent meeting of the International Society for Neutron Capture Therapy, BNCEFN was considered to be the most promising approach to further improvements in the treatment of brain tumors. It has the potential to significantly improve survival time for brain tumor patients.

(Supported by internal funding)

217. Detector Development for Boron Neutron Capture Therapy (BNCT) Dosimetry

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The Neutron Therapy Facility (NTF) plans to implement BNCT-enhanced fast neutron therapy for treating advanced brain tumors (glioblastoma multiformae). It is developing boron-loaded ionization chambers and associated gas and electronic systems to measure doses. This work is part of an international collaboration to develop a system that is easy to use, standardized, similar to conventional dosimetry, and acceptable to the conventional radiation therapy community.

(Supported by internal funding)

218. Radiobiological Studies of Dose Enhancement as a Function of Boron-10 Concentration in Brain Tumor Cells Treated in the Modified NTF Beam

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In collaboration with Rush University, The Neutron Therapy Facility (NTF) is preparing to measure cell survival curves for human glioma cell lines containing boron-10 and irradiated in the low-energy enhanced clinical fast neutron beam. Results will be directly relevant to the design of human clinical trials, treating advanced brain tumors.

(Supported by internal funding)

219. High Purity, Ultrafine Particle Production from Supercritical Fluids

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Many developing medicinal compounds capable of significantly improving human health require size reduction to micron and sub-micron dimensions. This manipulation of particle size provides a unique set of chemical and physical properties, including enhanced rate of biological uptake. Currently, the majority of identified pharmaceutical candidates have such low biological uptake rates that they are unusable. Low bioavailability is often the result of limited dissolution rates of the chemical through the gastrointestinal or pulmonary tract. Reducing particle size to micron and sub-micron dimensions can significantly increase the uptake rate such that previously unusable compounds can become viable, highly effective products. Although many conventional techniques, including hammer milling, ball milling, and jet milling, are available, they are

thermally opposing and difficult to control and reproduce. Thermally labile organic materials are unsuited for high-temperature processing methods, such as developing plasma methods. This LDRD project is developing a Supercritical Fluid (SCF) process that shows promise in the area of producing high-purity, ultrafine particles. The specific technical goal for this project is to develop SCF processes and nozzles that can yield a reproducible, controlled particle size distribution at production scales. We are working to develop a fundamental understanding of the dynamics that give rise to the control of particle size formation and parameters required for nozzle designs under varying operating conditions and particle size requirements. A multi-disciplinary group with the skills required to address these issues has been assembled at the INEEL. The INEEL team has expertise in nozzle design, numerical modeling, SCFs, and nano particles. The combined expertise of the INEEL team and a pharmaceutical industry partner will be brought to bear on this new technology area to elevate this emerging process to a production scale technology capable of significantly improving medicinal compound properties for the purpose of positively impacting human health.

[Supported by internal funding (LDRD)]

220. Endovascular Photo-Acoustic Recanalization (EPAR)

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Researchers at LLNL have developed unique technologies to aid in the disruption of thrombus occlusions for treatment of acute stroke. This minimally invasive technique, Endovascular Photo-Acoustic Recanalization (EPAR), involves guiding a catheter to the site of the occlusion and introducing an optical fiber delivery system into the catheter. High repetition rate laser light, delivered through the optical fiber emulsifies the clot via acoustic energy. The technology was licensed to EndoVasix, Inc. in November of 1996. EndoVasix has tested the device in animal studies, which showed positive results. EndoVasix will further advance designs for eventual investigations in humans in Europe in 1998.

(Supported by LLNL internal funding / Industry)

221. Silicon Microgripper

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Designers of tools for surgeons performing modern minimally-invasive surgery are challenged to create miniaturized systems to hold, grab and release materials, often at the end of a multi-meter length catheter deep within a patient's arterial system. Research engineers at the Lawrence Livermore National Laboratory have developed a silicon microgripper that is actuated by shape memory alloy (SMA) thin films. This microgripper device can be used for remote manipulation in small areas via access through small holes or catheters. This device can also be used in conjunction with other microtools to go through a singular trocar in laparoscopic procedures and reduce the amount of incisions required and cut down on the exchange of tools. Our microgripper generates a large gripping force (13mN), has a relatively rigid structural body and is designed with small cross-sectional areas to facilitate entry through small holes.

(Supported by LLNL internal funding / Industry)

222. X-Ray Catheter for Prevention of Restenosis

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Researchers at the Lawrence Livermore National Laboratory are miniaturizing a cold cathode x-ray source for the treatment of restenosis. The device is

catheter-based, and is used following balloon angioplasty to remove obstruction (stenosis) of coronary arteries. It has been shown that radiation to the arterial wall after angioplasty is effective in preventing the subsequent re-obstruction (restenosis) of the artery by the proliferation of smooth muscle cells. Radiation reduces the proliferation thereby preventing restenosis. Current treatments use radioactive pellets that are fed through the catheter to the site of the angioplasty. The x-ray catheter has the advantage of producing ionizing radiation only when energized within the artery at the site of the angioplasty. The prototype 3 mm diameter x-ray catheter was shown to produce therapeutic doses. Further miniaturization by the industrial partner is under way.

(Supported by Industry)

223. Medical Radioisotope Research

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The Medical Radioisotope Research Program at Los Alamos exists to support research and development into the application of radioactive nuclides for medical diagnosis and therapy. Personnel in the program have projects for the development of methods for accelerator production of promising radioactive materials to provide to researchers in Universities and Hospitals external to the Laboratory. This work is being funded through the DOE NE Isotope Program Office. Furthermore, using both past and present support from OBER we have collaborative projects to develop both diagnostic and therapeutic applications of radionuclides. The project presently funded through OBER involves the development of the Se-72/As-72 generator system for Positron Emission Tomographic Imaging for oncology and the development of arsenic-labeled radiopharmaceuticals for both imaging and therapy. We are also developing proposals to OBER and NIH relating to the application of radionuclides to molecular nuclear medicine.

[Supported by DOE (NE) Isotope Program Office, OBER, and a CRADA with Commercialization Technology International in Albuquerque]

224. Disinfection of Drinking Water

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Chlorination of the U.S. water supply generates many toxic chlorinated side-products that remain in the drinking water. Since all known biological cells (including bacteria, spores, phage, and viruses) have a net negative surface charge at neutral pH, end-use ion exchange resins (e.g. DEAE-cellulose) have been employed in some small communities to disinfect drinking water. However, chemical ion exchange resins are prohibitively expensive for routine use in larger communities since adsorbed microbes cannot be readily released from the resin, and it must be discarded after a single use. In an NREL-patented technology, electrode surfaces have been derivatized with electroactive groups which confer a positive electrode surface charge when oxidized (e.g., many ferrocenes, viologens, phenazines, oxazines, etc.) and therefore adsorb biological materials. Conducting beads (1-mm diameter) of graphite or ITO have been derivatized with electroactive groups and stacked in columns. Each cubic centimeter of bed volume has sufficient surface area to treat 650 liters of water containing 4000 bacteria per ml. The column can be taken off line, and, with the imposition of a small voltage to reduce the electroactive groups to anions, the negatively charged microbes are sloughed into the void volume and rinsed to the sewer. Upon reoxidizing the cleansed electrode surface, the column is ready to begin the next cycle of disinfection. Electrochemical control of this basic ion exchange system permits adsorption and desorption of biological materials at accurately controlled redox potentials, does not require ionic strength or pH adulterants, and can be easily automated. The technology should benefit public health by decreasing the need for high chlorination levels at the site of municipal water treatment and improve drinking water purity when employed at the end use. Other public health applications may include disinfecting air conditioning circulatory tanks, swimming pools and spas, and purifying hospital air.

(Supported by Spin-off from EERE Hydrogen Program)

225. The Development of Novel TiO_2 Photocatalytic Materials for Disinfection

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It is well documented that titanium dioxide (TiO_2) exhibits photocatalytic biocidal action by participating in chemical reactions to kill contacting microorganisms including bacteria, fungi, virus and cancer cells. The mineralization of microbes (i.e., total oxidation to CO_2 and other inorganic compounds) after long-term photocatalytic reaction has also been established by the authors. Thus TiO_2-coated media can serve as a self-sterilizing and self-cleaning filter for removal of bioaerosols from air. This would be a great advantage over conventional air filter systems where the used filter can provide an environment for pathogenic microbes to populate. Improvement of photocatalyst performance is a key to developing more effective air cleaning systems and applications for self cleaning surfaces. Both configurations have immediate application to the prevention of the spread of infectious agents in health maintenance facilities. In order to achieve higher killing efficiency on a large scale, new catalyst materials need to be developed to provide for better trapping, killing and mineralization of microbes. Research groups in the Center for Basic Sciences at NREL have expertise in fabricating new TiO_2-derived materials for application in solar cells. There is significance in the required functionality for the two different applications. This provides a rich source of new materials to be tested for photocatalytic biocidal activity. The multidisciplinary team environment at NREL facilitates the advancement of development in this important area. Developing and testing new materials for enhanced activity and their incorporation into new reactor design will facilitate the development and widespread use of this novel filter system in maintaining the well being of human health.

(Supported by NREL FIRST program, Center for Indoor Air Research, NASA, and the DOE Building Energy Technology, Hybrid Vehicle, and Initiative for Proliferation Prevention Programs)

226. Cancer Therapy with Radioisotopes Targeted to Tumor Blood Vessels

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Targeting molecules directed to tumor cells can theoretically be used to deliver toxic agents such as radioisotopes directly to tumor cells to kill them specifically. The process works very well for tumor cells such as leukemias where the target cells are found in the circulation. For carcinomas (solid tumors) there is an anatomical barrier (the blood vessel wall) which limits the amount of antibody which can bind to the tumor cells directly. The limitation is largely due to the relatively large size of the antibody protein. Experiments to counteract this problem include: (1) two step targeting whereby a second (small) molecule is coupled to the radioisotope and it then binds to the antibody which has already been parked at the tumor site and (2) protein engineering to make the final targeting molecule (engineered antibody) smaller so that more of the toxic agent will get to the tumor faster. Another approach to the blood vessel barrier problem is to target the tumor blood vessels themselves. This way, the large antibody doesn't need to get through the vessel wall. This approach has the potential to increase targeting efficiency 100 fold. The radioisotope bound to the antibody parked in the tumor blood vessel can irradiate through the wall and kill not only the tumor vessel cells and thus the blood supply but also kill tumor cells in the area. In a model system, we have shown that the α-particle emitter, ^{213}Bi, targeted to lung blood vessels can cure up to 100% of small lung tumors. Cured animals have life spans 5-10 times longer than control treated animals. These experiments show that α-particle emitters have excellent potential in therapy of small tumors, especially when targeted to blood vessels serving the tumors. General application of this technology will depend on finding the antibodies (or other targeting molecules) that can recognize and bind to tumor blood vessels and not normal blood vessels. Initial experiments using a molecular biology approach, phage display, indicate that unique targeting molecules can be identified. We have undertaken a

series of phage display library selections in model tumor systems to identify targeting molecules specifically reactive with tumor vasculature. The data indicate that libraries of scFv proteins hold the most promise for identification of specific targeting molecules.

(Supported by DOE OBER)

227. Develop and Test Injectable Radioisotope Polymer Composites for Cancer Therapy

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The objective of this project is to further develop and optimize therapeutic radionuclide polymer composites for cancer treatment. Directly injectable radionuclide polymer composites may be able to deliver highly localized, high-intensity radiation absorbed doses to solid tumors while minimizing radiation doses to normal tissues. These materials are expected to have several important applications in the treatment of non-resectable or radioresistant cancer as well as advantages over other forms of experimental infusional brachytherapy. Examples include therapy of prostate tumors (as an improved modality over permanent palladium-103 or iodine-125 seeds), brain tumors, pancreatic cancer, and therapy of breast cancer (as an improved modality over iridium-192 wire implants). Prior studies with temperature-sensitive gels have shown that the gels can be administered in liquid form, perfuse interstitial space, polymerize (within seconds) at body temperature (37°C), and contain radionuclides without appreciable leakage. The result is expected to be a more uniform, higher-dose therapy than can be achieved with conventional radiation therapy. Biodegradable gels are proposed for future investigation.

(Supported by DOE OBER)

228. Thermoreversible Polymeric Gels as Drug Delivery Systems for Chemo-Embolization Therapy of Hepatocellular Carcinoma

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This project involves development of a new drug delivery system utilizing stimuli-sensitive polymers. Specifically, a new class of chemo-embolic materials based on thermoreversible polymer gels will be developed and evaluated. The proposed drug delivery system is composed of thermoreversible polymer gel and an anticancer agent. The unique aspect of this novel system is that at body temperature the polymer forms a thermoreversible gel matrix that is able to entrap any anticancer agent. At room temperature the polymer is a free-flowing (injectible) solution which may be delivered locally to a desired site. The localized and controlled release of the anticancer agent entrapped within the thermoreversible gel matrix is expected to decrease the toxic effects of chemotherapy. The primary objective of this research is to develop a new drug delivery system based on thermally reversible polymers for the chemo-embolization therapy that will enhance the efficacy and decrease the systemic toxic effects of chemotherapy.

(Supported by PNNL LDRD funds)

229. Radium-223 Immunoconjugates for Cancer Therapy

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The purpose of this project is to prepare and test an alpha-emitting antibody as a potential therapeutic radiopharmaceutical. The main challenge has been to assemble and test individual components needed for the immunoconjugate. Any radioisotope used in

radioimmunotherapy must be chemically attached to the antibody. However, radium is one of the most difficult elements to complex and link to antibodies. A second challenge has been to form a stable chemical link between the ligand and the antibody. The next challenge was to find a suitable linker between the various candidate ligands and the antibody protein. Ligands were modified by adding either an amino or an isothiocyanatobenzyl functional group corresponding to the linker chemistry.

(Supported by PNNL LDRD funds)

230. Oxygen Generation for Medical Applications

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The logistics and cost of supplying medical grade oxygen to military medical units deployed to support troops involved in training exercises or combat operations is significant. The current standard of practice is to generate medical grade oxygen in large, fixed facilities and then distribute it, pressurized or liquefied, in various sized metal devices or pressurized cylinders. Recognizing the distinct advantages that a portable oxygen generator could offer to those responsible for providing care and support to soldiers in the field, this research and development project is using solid oxide electrolytes to separate oxygen from air. PNNL developed a prototype portable oxygen generator capable of producing pressurized medical grade oxygen. Proposed follow on work would focus on further development of the system beyond the prototype stage and investigation of new membrane materials that may enable reducing systems size and allow lower temperature operation.

(Supported by Army Medical Research and Materiel Command)

231. Ion-Induced Nuclear Radiotherapy—INRT

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The INRT concept is a new highly localized form of proton radiotherapy for cancerous tumors. INRT uses moderate energy (and cheap to generate) He-3 beams to produce 17 MeV protons through the D(He-3,p) fusion reaction at the end of a modified hypodermic needle. This needle is then injected to the site of a tumor to treat a 6-mm-diameter region in a matter of minutes. With the LDRD project, we successfully performed dosimetry on the radiation field produced by the fusion protons, developed a computer model of the 3D-dose distribution, and ultimately demonstrated the feasibility of the INRT concept. This is an inexpensive alternative to proton radiotherapy, which has an added advantage of significantly reduced collateral damage to healthy tissues. Time frame: LDRD FY96, patent issued FY97, and licenses FY98.

(Supported by Laboratory Directed Research & Development)

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