Laboratory Directed Research & Development Program

Annual Report to the Department of Energy
December 1994

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BROOKHAVEN NATIONAL LABORATORY
ASSOCIATED UNIVERSITIES, INC.
UPTON, NEW YORK 11973-5000

UNDER CONTRACT NO. DE-AC02-76CH00016

UNITED STATES DEPARTMENT OF ENERGY

MASTER

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Printed in the United States of America
Available from
National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road
Springfield, VA 22161

NTIS price codes:
Printed Copy: A06; Microfiche Copy: A01

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Acknowledgments

The Laboratory Directed Research and Development (LDRD) Program is directed by Martin Blume, Deputy Director and is administered by Henry Grahn, Associate Director for Administration (ADA). Preparation of the FY 1994 report was coordinated and edited by Gregory Ogeka and Anthony Romano, Special Assistants to the ADA who wish to thank Virginia Morante for her assistance in organizing and typing the document. A special thank you is also extended to Beth Blevins and Susan Cuevas for their help in proof-reading sections of the report and to the Photography and Graphic Arts Division for their help in publishing. Of course, a very special acknowledgement is extended to all of the authors of the project annual reports, and to their secretaries.

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Brookhaven National Laboratory

Past

***O*n the previous page:**

Brookhaven National Laboratory in 1948. A view from the north showing the many Army barracks buildings, formerly part of Camp Upton, which would become the foundation upon which the Laboratory would develop.

Introduction

Background: Brookhaven National Laboratory (BNL) was established in 1947 on the site of the former Army Camp Upton. Brookhaven is a multidisciplinary laboratory that carries out basic and applied research in the physical, biomedical and environmental sciences, and in selected energy technologies. The Laboratory is managed by Associated Universities, Inc., under contract with the U. S. Department of Energy. BNL's annual budget has averaged about \$380 million, and our facilities are valued at over \$2.2 billion. There are about 3,300 employees, and another 4,000 guest scientists and students come each year to use the Laboratory's facilities and work with our staff. BNL's Relativistic Heavy Ion Collider (RHIC), presently under construction, will be the world's foremost facility for nuclear physics research. RHIC will create the hot, dense plasma of quarks and gluons from which particles condensed after the "Big Bang" of the early universe.

Mission and Core Competencies: Brookhaven National Laboratory's mission is to support the basic Department of Energy (DOE) activities through our research and technology development, our educational efforts, and industrial involvement. Brookhaven was founded as a laboratory which would provide specialized research facilities that could not be designed, built and operated at a university or industrial complex, and this still remains a basic mission of the Laboratory. Brookhaven National Laboratory has four core competencies.

Brookhaven's four core competencies, Research Facilities, Scientific Research, Technology Development, and Knowledge Transfer are not independent isolated competencies. They are interrelated in a complex manner.

MAJOR CORE COMPETENCIES

RESEARCH FACILITIES

Expertise to conceive, design, build and operate complex leading-edge, user-oriented research facilities in a safe and environmentally responsible manner.

SCIENTIFIC RESEARCH

Expertise to carry out basic and applied scientific research in long-term, high-risk programs. This is an essential capability needed to keep our research facilities at the leading edge. These programs lead to new insights and technological advances which provide the underlying scientific base for the DOE missions and generate long-term benefit to the nation.

TECHNOLOGY DEVELOPMENT

Expertise to develop advanced technologies that address national needs, support and strengthen the ability of DOE to carry out its missions, support other federal and state agencies, and enable industry to benefit from the multidisciplinary research and development at the Laboratory.

KNOWLEDGE TRANSFER

Expertise and mechanisms for disseminating scientific and technical knowledge to educate new generations of scientists and engineers to produce a technically trained workforce, to enhance scientific literacy of the general public, and to improve the competitiveness of U. S. industry.

Research Facilities and Scientific Research have a synergistic relationship. To maintain and constantly improve a research facility, and to keep it at the cutting edge, it is essential that the Laboratory have a significant research staff of excellent stature. The staff will drive the performance of the facility. Having the several complementary facilities at one location, such as the National Synchrotron Light Source and the High Flux Beam Reactor, allows a unique research capability, such as in material science and biological structure determination. The other two core competencies, Technology Development and Knowledge Transfer, bridges all of the research facilities and research programs.

Brookhaven's core competencies support and cut across the five central activities of the Department of Energy, as defined in its Strategic Plan. BNL plays a major role in the Science and Technology, the Environmental Quality, the Industrial Competitiveness and the Energy Resources sectors, with a smaller, but special role in the National Security arena. In order to better see the connection between the various Brookhaven activities that form the core competencies and the Department of Energy

central activities, each broad competency is with the following letter code describing letter code describing the match.

DOE Strategic Plan Activities	
SCI	Science and Technology
ENV	Environmental Quality
IND	Industrial Competitiveness
ENER	Energy Resources
SEC	National Security

DOE ACTIVITIES

	Science and Technology	Environmental Quality	Energy Resources	Industrial Competitiveness	National Security
Research Facilities					
• Alternating Gradient Synchrotron	X				
• Relativistic Heavy Ion Collider (under construction)	X				
• High Flux Beam Reactor	X	X	X	X	
• National Synchrotron Light Source	X	X	X	X	
• Pet-Cyclotron Center	X			X	
• Brookhaven Linac Isotope Production Facility	X			X	
• Medical Radiation Facility	X			X	
• Brookhaven Medical Research Reactor	X			X	
• Scanning Transmission Electron Microscope	X			X	
• Protein Data Bank	X			X	
• Genome Sequencing Center (under development)	X			X	
• Proton Radiation Therapy Facility (under development)	X	X	X	X	X
• Tandem Van De Graaff Facility	X			X	X
• Accelerator Test Facility	X	X	X		
• 10 MeV Electron Pulse Radiolysis Facility (under construction)	X			X	X
• Nuclear Data Compilation and Evaluation Center					
Scientific Research					
• High Energy Particle and Nuclear Physics	X				X
• Advanced Accelerator Concepts	X			X	
• Materials Sciences	X		X	X	
• Chemical Sciences	X	X	X	X	
• Environmental Sciences	X	X		X	
• Medical Science	X			X	
• Molecular Biology and Biotechnology	X			X	
• Advanced Scientific Computing and Systems Analysis	X	X	X	X	
Technology Development					
• Physical, Chemical and Materials Science	X		X	X	
• Accelerator Technology	X			X	X
• Environmental and Conservation Technologies	X	X	X	X	X
• Medical Technologies	X			X	
• Biotechnology	X			X	
• Safety and Risk Assessment	X	X	X		X
Knowledge Transfer					
• Educating Future Generations of Scientists	X	X	X	X	X
• Educating the General Public	X	X	X	X	X
• Technology Transfer to Industry	X	X	X	X	X

MAJOR ACTIVITY CLUSTERS

LARGE RESEARCH FACILITIES

ALTERNATING GRADIENT SYNCHROTRON

(SCI)

- Research in Particle and Nuclear Physics
- High-Intensity Frontier of Particle Physics
- World's Only High Energy Polarized Proton Source
- At Present, Nation's Only High Energy, Heavy Ion Synchrotron
- Over 800 Users

RELATIVISTIC HEAVY ION COLLIDER

(under construction)

(SCI)

- High-Temperature Frontier of Nuclear Matter
- Large and Unique High Energy Physics Potential (e.g. spin physics)
- 600 Users in First Round Experiments

HIGH FLUX BEAM REACTOR

(SCI, ENER, ENV, IND)

- Instruments for Research in Condensed-Matter Physics, Biology, Chemistry, Applied Sciences and Industrial Applications
- 270 Users

NATIONAL SYNCHROTRON LIGHT SOURCE

(SCI, ENV, ENER, IND)

- Two Storage Rings Providing Intense UV and X-ray Photon Sources
- Instruments for Research in Materials Science, Biology, Chemistry, Medical and Industrial Applications
- Over 3300 Users, Including 400 Industrial Users

BIOMEDICAL FACILITIES

PET-CYCLOTRON CENTER

(SCI, IND)

- Medical Imaging for Basic Neuroscience and Substance Abuse, Radiotracer Synthesis

BROOKHAVEN LINEAR ISOTOPE PRODUCTION FACILITY

(SCI, IND)

- Production of Isotopes for Medical Purposes
- Approximately 200 Isotopes Produced for Commercial and/or Research Use

MEDICAL RADIATION FACILITY

(SCI, IND)

- Cancer Patient Treatment: 250 patients annually

BROOKHAVEN MEDICAL RESEARCH REACTOR

(SCI, IND)

- Structural Biology, Molecular Masses
- Over 75 Users

PROTEIN DATA BANK

(SCI, IND)

- World-Wide Repository for Three-Dimensional Structures of Biological Macromolecules

GENOME SEQUENCING CENTER

(under development) (SCI, IND)

- Large-Scale DNA Sequencing

PROTON RADIATION THERAPY

FACILITY (under development)

(SCI, IND)

- Utilizes Existing 200 MeV Linac
- Will Consist of Horizontal and Vertical Beam Treatment Rooms
- Capability of 900 Cancer Patients Per Year

OTHER FACILITIES

TANDEM VAN DE GRAAFF FACILITY

(SCI, SEC, ENV, ENER, IND)

- Microchip Radiation Testing Facility
- Film Irradiation Plant for Track Etching Filter Membranes
- 250 Industrial Users from 45 Institutions

ACCELERATOR TEST FACILITY

(SCI, IND, SEC)

- Advanced Acceleration Concepts

10-MEV ELECTRON PULSE

RADIOLYSIS FACILITY

(under construction)

(SCI, ENER, ENV.)

- Study of Rapid Chemical Reactions: Catalysis, Energy Conversion and Storage

NUCLEAR DATA COMPILATION AND EVALUATION CENTER

(SCI, SEC, IND)

- Nuclear Cross-Section and Structure Data
- 1100 Users

SCIENTIFIC RESEARCH

HIGH ENERGY PARTICLE AND NUCLEAR PHYSICS

(SCI)

- Beyond the Standard Model
 - Rare Kaon Decays
 - Muon Anomalous Magnetic Moment
 - Exotics and Glueball Spectroscopy
 - Neutrino Oscillation
 - Strange Matter
 - Solar Neutrinos
- Relativistic Heavy Ions
 - High-Temperature Nuclear Matter
 - Quark-Gluon Plasma

ADVANCED ACCELERATOR CONCEPTS

(SCI, IND, SEC)

- Short Wavelength Accelerating Structures
- Production of Coherent Radiation Free Electron Laser

MATERIALS SCIENCES

(SCI, ENER, IND, ENV)

- Materials Characterization with Neutron and X-ray Scattering, Magnetism and Superconductivity, Surface Studies, Corrosion, Adhesion, Catalysis, Metallic Alloys, Polymers

CHEMICAL SCIENCES

(SCI, ENER, IND, ENV)

- Molecular Dynamics, Reactive Transient Species, Thermal and Photo-Induced Charge Transfer, Structure and Reactivity

ENVIRONMENTAL SCIENCES
(SCI, ENV, IND)

- Global Change, Atmospheric Chemistry, Oceanography, Soil Chemistry, Cycling of Pollutants, Environmental Remediation

MEDICAL SCIENCE
(SCI, IND)

- Medical Imaging: PET, SPECT, MRI, Coronary Angiography
- Nuclear Medicine
- Radionuclides, Radiopharmaceuticals, Synthesis and Applications
- Advanced Cancer Therapies: Neutron Capture, Microbeam Radiation, Proton Radiation
- Mechanisms of Oncogenesis

MOLECULAR BIOLOGY AND BIOTECHNOLOGY
(SCI, IND)

- Genome Structure, Gene Expression
- DNA Damage and Repair
- Molecular Genetics
- Plant Science
- Bio-Structure Determination by X-ray and Neutron Scattering
- Enzyme Kinetics by Laue Crystallography
- Mass Measurements by Electron Microscopy

ADVANCED SCIENTIFIC COMPUTING AND SYSTEMS ANALYSIS
(SCI, IND, ENV, ENER)

- Risk Assessment
- Energy Modeling
- Groundwater Modeling

TECHNOLOGY DEVELOPMENT

PHYSICAL, CHEMICAL AND MATERIALS SCIENCE
(SCI, ENER, IND)

- State-of-the-Art Instrumentation and Devices for Precision Electronics, Optics and Microelectronics
- Superconducting Materials
- X-ray Lithography
- Micromachining
- Battery Technology
- Permanent Magnets
- Smart Polymers

ACCELERATOR TECHNOLOGY
(SCI, IND, SEC)

- High-Field, High-Quality Superconducting Magnets
- High-Power Radio Frequency Systems
- Ultrahigh Vacuum Systems
- Advanced Accelerator Designs
 - High-Gradient Acceleration
 - High-Beam Current Acceleration

ENVIRONMENTAL AND CONSERVATION TECHNOLOGIES
(SCI, ENV, ENER, IND, SEC)

- Environmental Remediation and Mitigation
- Energy-Efficiency Technologies
- Waste Treatment
- Disposal of Nuclear Materials
- Radiation Protection
- Infrastructure Modernization
- Transportation: Intelligent Vehicle Highway System, MAGLEV

MEDICAL TECHNOLOGIES

(SCI, IND)

- Biomedical Applications of Nuclear Technology
- Production of Radionuclides and Radiopharmaceuticals
- Development of Particle Radiation Therapies for Cancer
- Medical Imaging
- X-ray Microbeam Therapy

BIOTECHNOLOGY

(SEC, SCI, ENV, ENER)

- Neutron and Synchrotron X-ray Scattering
- Large-Scale Genome Sequencing
- High-Resolution Scanning and Cryo Electron Microscopy
- Cloning, Expressing and Engineering Genes
- Metal Cluster Compounds for Electron Microscope Labels
- Phage Displays for Probing Specific Interactions

SAFETY AND RISK ASSESSMENT

(SEC, SCI, ENV, ENER)

- Safeguards, Nonproliferation and Arms Control
- Safety Analysis of Complex Systems
- Probabilistic Risk Assessment

KNOWLEDGE TRANSFER

EDUCATING FUTURE GENERATIONS OF SCIENTISTS AND ENGINEERS

(SCI, ENV, IND, ENER, SEC)

- Lecturing, Conference Participation
- Visiting Scientist Program
- Accelerator Fellowship Program
- Postdoctoral Research Associates
- Engineering Intern Program
- Graduate Student Thesis Projects
- Adjunct Teaching Appointments at Local Colleges
- Office of Educational Program
 - Precollege and College Programs for Students and Teachers

EDUCATING THE GENERAL PUBLIC

(SCI, ENV, IND, ENER, SEC)

- Science Museum and Laboratory Tours (20,000 people/year)
- Speakers Bureau
- BNL Videos
- Laboratory Lectures for the Public
- Community Outreach Programs
- Information Storage and Transfer

TECHNOLOGY TRANSFER TO INDUSTRY

(SCI, ENV, IND, ENER, SEC)

- Industrial Users at the Research Facilities
- Consulting by Scientific Staff
- Technology Transfer Office
 - Patenting and Licensing Office
 - Technical Assistance for Industry
 - CRADAs
 - Visiting Scientist Program with Industry
 - Research Partnerships with Industry
 - Industry-Sponsored Proprietary Research and Development
- Long Island Research Institute (LIRI) (founding member)
 - Promotes Laboratory-Industry Interaction
 - ARPA-Funded BNL/LIRI Defense Conversion Project
 - NY State-Funded Biotechnology Initiative

INFORMATION TRANSFER

(SCI, ENV, IND, ENER, SEC)

- INFORM - Electronic Information Source
- Networking - "Information Superhighway"
- Technical and Scientific Publishing
- National Nuclear Data Center
- Protein Data Bank

New ideas and opportunities, fostering the advancement of technology, are occurring at an every increasing rate. It, therefore, seems appropriate that a vehicle be available which

fosters the development of new ideas and technologies, promotes the early exploration and exploitation of creative and innovative concepts, and develops new "fundable" R&D projects and programs if BNL is to carry out its primary mission and support the basic Department of Energy activities. At Brookhaven National Laboratory, one such method is through its Laboratory Directed Research and Development Program. This discretionary research and development tool is critical in maintaining the scientific excellence and vitality of the Laboratory. Additionally, it is a means to stimulate the scientific community, fostering new

science and technology ideas, which is the major factor in achieving and maintaining staff excellence, and a means to address national needs, within the overall mission of the DOE and the BNL.

The Project Summaries, with their accomplishments described in this report, reflect the above. Aside from leading to new fundable or promising programs and producing especially noteworthy research, they have resulted in numerous publications in various professional and scientific journals, and presentations at meetings and forums.

Management Process

PROGRAM DESCRIPTION:

Introduction: The Department of Energy's (DOE) Laboratory Directed Research & Development (LDRD) Program at Brookhaven National Laboratory (BNL) was originally established as the "Exploratory Research Program" under the guidelines set forth in DOE Order 5000.1 in May 1984. From inception through September 1995, a period spanning eleven fiscal years, the Laboratory has authorized \$22.2 million in Exploratory R&D, consisting of 150 separate projects.

BNL LDRD PROGRAM HISTORY 1985-1995

FISCAL YEAR	AUTH K\$	COSTED K\$	NO. REC'D	NEW STARTS
1985	1,842	1,819	39	13
1986	2,552	2,515	22	15
1987	1,451	1,443	29	8
1988	1,545	1,510	46	14
1989	2,676	2,666	42	21
1990	2,008	1,941	47	9
1991	1,353	1,321	23	14
1992	1,892	1,865	30	14
1993	2,073	2,006	35	14
1994	2,334	2,323	44	15
1995	2,467	--	45	13
TOTALS	22,193	19,409	402	150

Historical Perspective: Brookhaven National Laboratory was established in 1946. Throughout its history, certain projects of an exploratory nature, sometimes referred to in the past as "seed money projects," were supported with overhead funding. In 1979, as a result of a Review Audit

in that year, the seed money accounting procedures were formalized, and oversight by the DOE Brookhaven Area Office Manager was first established. This seed money program operated at a variable level of funding, which averaged about 0.1 percent of the Laboratory's operating budget over the period 1979 to 1984.

In May 1984, the program was expanded. The expanded program embraced the new Exploratory R&D guidelines of DOE Order 5000.1. The new program, called the Exploratory Research Program, was put into effect for FY 1985 funding. The current Laboratory Directed Research & Development Program reflects the operating styles and many of the procedures of the earlier programs, which have evolved somewhat informally over the years. It also encompasses the requirements of the current DOE Order 5000.4A.

Goals and Objectives: The goals and objectives of BNL's LDRD Program can be inferred from the Program's stated purposes. These are to (1) encourage and support the development of new ideas and technology, (2) promote the early exploration and exploitation of creative and innovative concepts, and (3) develop new "fundable" R&D projects and programs. The emphasis is clearly articulated by BNL to be on supporting exploratory research "which could lead to new programs, projects, and directions" for the Laboratory.

General Characteristics of the LDRD Program: Projects or studies that are appropriate candidates for the Laboratory's LDRD Program include, but are not limited to, (1) projects, normally relatively small, in the forefront areas of basic and applied science and technology for the primary purpose of enriching laboratory capabilities, (2) advanced study of new hypotheses, new concepts, or innovative approaches to scientific or technical

problems, (3) experiments and analyses directed toward "proof of principle" or early determination of the utility of new scientific ideas, and (4) conception and preliminary technical analysis of experimental facilities or devices.

PROGRAM ADMINISTRATION:

Overall Coordination: Overall responsibility for coordination, oversight, and administration of BNL's LDRD Program resides with the Laboratory's Deputy Director. The Deputy Director is assisted in the administration of the program by the Office of the Associate Director for Administration, which administers the program budget, establishes the project accounts, maintains summary reports, and reports Program activities to the DOE through the Brookhaven Area Office Manager.

Responsibility for the allocation of resources and the orchestration, review and selection of proposals, lies with a top-level group, called the Laboratory Directed Research & Development Committee. The Committee is made up of seven members. The Laboratory's Deputy Director is the chairperson of the Committee. The other members are the Associate and Assistant Directors of the Laboratory.

LDRD PROGRAM COMMITTEE

Martin Blume	Chairperson
John D. Axe	Basic Energy Sciences
Seymour Baron	Applied Programs
Henry C. Grah	Administration
Mark Sakitt	Planning & Policy
Richard B. Setlow	Life Sciences
Laurence Trueman	High Energy & Nuclear Physics

Allocating Funds: There are two types of decisions to be made each year concerning the allocation of funds for the LDRD Program. These are (1) how

much money should be budgeted overall for the Program; and (2) of this, how much, if any, should go to each competing project or proposal. Both of these decisions are made by top-level management.

Concerning the overall budget, for each upcoming fiscal year the Laboratory Director, in consultation with the Deputy Director and the Associate Director for Administration, develops an overall level of funding for the LDRD Program. The budget amount is then incorporated into the Laboratory's LDRD Plan which formally requests authorization from the DOE to expend funds for the LDRD Program up to this ceiling amount.

The actual level of funding available for LDRD, however, may turn out to be much less than this ceiling. The actual level is determined during the course of the year and is affected by several considerations, including: the specific merits of the various project proposals, as determined by Laboratory management and the members of the LDRD Program Committee; the overall financial health of the Laboratory; and a number of budgetary tradeoffs between LDRD and other overhead expenses.

LDRD COSTS VS. TOTAL LABORATORY COSTS

\$ in millions					
FISCAL YEAR	DOE FUNDS	WFO FUNDS	TOTAL FUNDS	LDRD FUNDS	% OF TOTAL
1985	153.0	40.4	193.1	1.82	0.9
1986	156.5	45.1	201.6	2.52	1.2
1987	161.7	45.6	207.3	1.44	0.7
1988	176.7	45.9	222.6	1.51	0.7
1989	193.6	46.7	240.3	2.67	1.1
1990	203.8	45.2	249.0	1.94	0.8
1991	220.9	50.3	271.2	1.32	0.5
1992	234.3	47.2	281.5	1.87	0.7
1993	231.4	47.3	278.7	2.01	0.7
1994	252.0	45.4	297.1	2.32	0.8

Concerning the allocation of resources to specific topic areas or to individual project proposals, such issues are addressed on a case-by-case basis by the LDRD Program Committee, once specific proposals have been received. The Committee meets periodically to review and recommend project proposals, and to determine the amount of funding to be made available to the LDRD Program. The requirements of DOE Order 5000.4A are carefully considered during the selection process to ensure that proposals are consistent with DOE's criteria.

PROGRAM PROCESS

Request for Proposals: The availability of special funds for research under the LDRD Program is well publicized throughout the Laboratory. This is done using two methods, one occurring at yearly intervals, the other occurring irregularly. Each year in May a memo is sent by the Laboratory Director to all scientific staff, issuing a "call for proposals." This memo is accompanied by an attached document, entitled "Guidelines and Procedures for Developing Proposals

via the Laboratory Directed Research and Development (LDRD) Program." The other method is by announcement in the Brookhaven Bulletin, the Laboratory's weekly newspaper; but the nature of the announcements varies and they appear at irregular intervals. In some years the Bulletin prints an article that amounts to a separate call for proposals. In other years the Bulletin publishes articles on specific research projects which, in effect, help advertise the LDRD Program.

The "Guidelines and Procedures" document specifies the requirements necessary for participation in the program. It states the program's purpose, general characteristics, procedures for applying, and restrictions. An application for funding, that is, a project proposal, takes the form of a completed "Proposal Questionnaire." An application must be approved up the chain-of-command, by the initiator's Department Chairman or Division Head, and by the cognizant Associate Director. Plans to ensure the satisfactory continuation of the principal investigator's regularly funded programs must also be approved. The applications are then forwarded to the Chairperson of the LDRD Program Committee for further review and consideration for funding.

The process which solicits and encourages the development of proposals has evolved into two modes of operation. Specifically, the ideas for proposal development may originate among the scientific staff in response to the general call for proposals. Alternatively, they may be initiated by top-level Laboratory management. Eventually, both follow the standard procedure for proposal approval, up the chain-of-command to the same decision makers. The fact that all proposals must be approved up the chain-of-command permits BNL managers to consider all ideas together when designing the mix of projects for the LDRD Program.

An initiative from management typically takes the form of a general topic area or item of special interest. It is not a directive, nor is it

included in the call for proposals, but the idea is communicated to a group of scientific staff, which is known to be in a position capable of pursuing and developing the idea in the form of a more formal proposal.

Proposal Review: Once a proposal is approved by the cognizant line managers, all proposals are forwarded to the Chairperson of the Committee who transmits a copy of all proposals received to the Committee for review. The Committee considers all proposals that have met certain minimum requirements pertaining to the Department's and BNL's LDRD policies.

Lead responsibility for the review of a proposal is then assigned to that member of the Committee who last approved it in the chain-of-command, that is, the member who oversees and directs the technical area from which the proposal originated. All members have several weeks to review the proposal and prepare for the next Committee meeting. During this time, additional reviews, if desired, may be arranged.

Formal peer reviews, consisting of written comments by experts outside the normal lines of supervision, are not usually performed. The members of the Committee are considered to have sufficient technical knowledge so that peer reviews are seldom required.

At the next Committee meeting, the Committee member responsible for the review of the proposal presents the proposal to the other members of the Committee. This is done without the member necessarily becoming an advocate for the proposed project.

Selection Criteria: Before proposals can be considered by the Committee, they must be screened to ensure that they meet a set of minimum requirements concerning the Department's LDRD policies and the Laboratory's own guidelines.

Minimum requirements of each proposal

are: (1) consistency with program purpose; (2) consistency with missions of BNL, DOE, and NRC; (3) approval by Department Chairman and/or Division Head, and cognizant Associate Director; (4) assurance of satisfactory continuation of principal investigator's regularly funded programs; (5) modest size and limited duration; (6) will not substitute for, supplement, or extend funding for tasks normally funded by DOE, NRC, or other users of the Laboratory; (7) will not require the acquisition of permanent staff; (8) will not create a commitment of future multi-year funding to reach a useful stage of completion; and (9) will not fund construction line-item projects, facility maintenance, or general purpose capital equipment.

The selection criteria used to evaluate and rank individual proposals are not formally stated or published. While the "Guidelines and Procedures" document clearly states that "awards will be made on a competitive basis," the factors or selection criteria to be considered in this competition are not listed. Nevertheless, selection is based on (1) scientific merit, (2) compliance with minimum requirements, (3) proposal cost as compared to the amount of available funding, (4) innovativeness, and (5) its potential for follow-on funding. The requirements of DOE Order 5000.4A are also carefully considered during the selection process to ensure that proposals are consistent with DOE criteria.

Project Approval: After all presentations are heard, the Committee attempts to arrive at a consensus concerning the highest priority proposals. Differences, if any, are resolved by the Chairperson. Also, a balance is struck between the prevailing financial needs of the Laboratory, which may vary over the course of the year, and the priorities of the projects considered. Some funding is held in reserve during the earlier meetings of the fiscal year so that funds remain available for proposals submitted at later dates. The funding amount requested in any one specific proposal may be

changed or adjusted during the approval process. The Committee's recommendation is then submitted to the Director for his approval.

The Associate Director for Administration is then notified, so that a separate Laboratory overhead account can be established to budget and collect the costs for the project. Statistics on the number of projects approved, compared to those rejected, show an overall approval rate of about 37 percent for new starts. From inception of the program through September 1994, 402 project proposals were considered and 150 were approved. Seven scientific departments and the RHIC Project were represented in the FY 1994 LDRD Program.

Project Supervision: Supervision over the actual performance of LDRD projects is carried out in the same way as other research projects at the Laboratory. Each principal investigator is

itself is carried out according to standard expectations of professionalism and scientific method. The manager is kept informed of the project's status, schedule, and progress.

The manager ensures that the work is completed in a timely manner and that annual status reports are submitted to the Deputy Director. In addition, LDRD Program activity is reported to the DOE Area Office Manager, including copies of all funded proposals, a LDRD Program data base, and a project funding and schedule summary report.

Project Reporting: Routine documentation of each project funded under the LDRD Program consists of a file containing: (1) a copy of the written proposal; (2) all interim status reports; (3) notifications of changes in research direction, if any; and (4) reports on cost incurred. Also, a formal Annual Report on the LDRD Program is submitted to BNL management and the DOE, summarizing work progress, accomplishments, and project status on all projects.

Documentation for the overall Program consists of (1) various program history files; (2) a running list of all proposals with their acceptance/rejection status, (3) funding schedule and summary reports for all approved projects, (4) permanent records on cost accounting, and a data base containing information on each funded project (description, funding by fiscal year, status and accomplishments, follow-on funding, publications, etc.).

Some of the projects involve animals or humans. Those projects have received approval from the Laboratory's appropriate review committees. The projects which involve animals or humans are identified in this report as follows:

Note: Those projects which involve animal vertebrates or human subjects have been so identified in the text.

assigned to an organizational unit (Department, Division), which is supervised by a manager.

Each manager is responsible for seeing that the obligations of the principal investigator are satisfactorily fulfilled and that the research

Broad Technical and Scientific Categories

Over the past several years, BNL has been categorizing its LDRD programs into six broad technical and scientific areas, including one miscellaneous area. The programs targeted for funding by BNL's LDRD Program in FY 1994 fall into the following broad technical and scientific categories:

New Directions for Energy Technologies: In the course of basic research efforts there are occasionally discoveries which hold promise for utilization in energy technologies. Such research is of significance, both for the Laboratory and for the DOE.

Global Change: BNL has had several programs in atmospheric, oceanographic, and mathematical sciences, which produce results of relevance to questions of global climate change and acid precipitation. LDRD proposals in these areas, which move in new avenues, are given priority as the Lab tries to make contributions to solutions of these problems. Calcification of marine alga (Project #93-10) is an example of such a program.

Radiation Therapies and Imaging: Applications of the Laboratory's facilities to the treatment of cancer and in imaging of the human body for diagnostic purposes, including such techniques as non-invasive coronary angiography, microplaner x-ray therapy, uranium neutron capture therapy and the use of other imaging techniques, are a significant part of the LDRD effort.

Genetic Studies: This area of research at the Laboratory has produced many new ideas and

applications, which exceed the capacity of DOE for support, and which are too important to remain undeveloped. LDRD funded projects in this are varied in subject matter covering many bio-medical topics.

New Directions for the Development and Utilization of BNL Facilities: High priority is assigned to ideas for more efficient utilization and for new directions for our major facilities. Ideas for more useful sources of x-rays at the synchrotron light source, for the utilization of new electron sources in the chemical study of pulse radiolysis, and new laser systems are all areas which are given priority for support.

Other Miscellaneous: Finally, the Program is open to significant and original ideas which do not necessarily fall within the bounds of the aforementioned priority areas. These usually involve small individual programs and projects that are judged to have high scientific/technical merit. Typically, materials development, electrochemical studies, advanced nuclear concepts and new bio-chemistry technologies fall into this category.

Brookhaven National Laboratory's FY 1994 LDRD Program covered the following Scientific and Technical Areas.

	Funding
Programs/Projects	\$000
New Directions for Energy	
Technologies	\$ 0
Global Change	160
Radiation Therapies and Imaging	613
Genetic Studies	344
New Directions for the	
Development and Utilization of	
BNL Facilities	780
Other - Miscellaneous	426
TOTAL	\$ 2,323

Brookhaven National Laboratory Present

On the previous page:

Brookhaven National Laboratory in 1993. An aerial view showing the various facilities which will be inter-connected to become the Relativistic Heavy Ion Collider (RHIC) complex. The user facilities shown in the photograph, some in operation and others under construction, represent part of the present and near-term future research of the Laboratory.

LABORATORY DIRECTED RESEARCH AND DEVELOPMENT
1994 PROJECT PROGRAM SUMMARIES

Effect of a Bacterial Spore Protein on Mutagenesis

J. K. Setlow

91-21

PROJECT DESCRIPTION:

Mutagenesis and killing caused by a spore protein from *Bacillus subtilis* that drastically alters DNA configuration are being studied in the bacterium *Escherichia coli*.

TECHNICAL PROGRESS AND RESULTS - Fiscal Year 1994:

Purpose: Since we have found that mutagenesis is substantially increased when DNA configuration is altered by the *B. subtilis* protein, the project involves investigation of details of the mechanism of the effect on mutation.

Approach: (a) In order to obtain evidence concerning the mechanism of the biological effects (mutagenesis and killing), we have begun to alter individual amino acids in the protein by site-directed mutagenesis, followed by assay of the biological effects. Eventually structural studies of these mutant proteins and their binding to DNA are expected to provide details on the mechanisms of these biological effects.

(b) Last year we found that *E. coli* containing the plasmid carrying the *B. subtilis* gene as a function of the number of cell divisions first lost the mutagenesis and later the killing. To explain the data we postulated that the *B. subtilis* gene itself was altered. This was wrong, as we found by sequencing the genes, which were all the same as the original wild

type. Furthermore, if the plasmid from cells which had lost the biological activity of the *B. subtilis* protein was removed and transferred into cells which had not previously been exposed to the protein, the biological properties were found still to be intact. Thus the changes we observed were in the bacteria, not the plasmid. We plan to clone the *E. coli* part of the chromosome that caused immunity to the *B. subtilis* protein with the use of a cosmid vector based on the *E. coli* F factor replicon.

(c) Having succeeded in making the Ripley system work for *in vivo* mutagenesis with the plasmid pPS708 carrying the *B. subtilis* gene and an M13 phage derivative, we will use the same system for *in vitro* mutagenesis studies to measure mistakes made with purified DNA polymerase and DNA and purified *B. subtilis* protein.

Technical Progress and Results: (a) We have constructed ten mutations in the *B. subtilis* gene coding for single amino acid changes in the protein, one with two changes and one with three. The principal result was that five of them showed high mutagenesis but little or no killing, all in one section of the gene. This was the clearest possible evidence that the mechanism of mutagenesis was completely different from that of the lethal effect. One of the mutant proteins caused more mutagenesis than the wild type, and two of them caused a disappearance of both mutagenic and lethal activity, and also failed to bind DNA *in vitro*.

(b) The first part of the cloning of *E. coli* immunity to the *B. subtilis* protein is to produce an immune cell which contains no plasmid. In the process we found that immune cells containing the plasmid grow considerably more slowly than normally. This defect may be related to the cell's immunity.

(c) The Ripley system was used for *in vivo* mutagenesis of derivatives of phage M13 containing inserts 74 base pairs long of the *rIIB* gene of T4 phage, along with the pPS708 plasmid in *E. coli* JM101, which can produce large amounts of the *B. subtilis* protein. There was an enormous mutational effect on the *rIIB* insert. Frameshifts within these inserts are detected because adjoining β -galactosidase sequences with altered frameshift, producing the wild-type reading frame, give a blue plaque with the addition of X-gal to the medium.

The reason for the large effect on the *rIIB* insert is probably the high A-T content of the

insert, a condition we previously found to be much more likely to be altered by the *B. subtilis* protein.

LDRD FUNDING:

FY 1991	\$ 87,461
FY 1992	159,594
FY 1993	161,915
FY 1994*	30,155

*This project's term was 3 years, starting in January 1991 and ending in December 1993.

Studies on the Cellular Toxicity of Cocaine and Cocaethylene

Albert J. Saubermann, 92-15
Nora D. Volkow and Joanna S. Fowler

PROJECT DESCRIPTION:

This project seeks to measure the effects of cocaine, cocaethylene, alcohol, and alcohol with cocaine on elemental distribution in the cells of the leech ganglia (neurons and glial cells). We hypothesize that the effects of cocaine and cocaethylene are equipotent in their ability to block Na^+ conductance into the cell and that the combination with alcohol will, in addition, disturb the intracellular concentration of Cl^- and Ca^{++} . This project utilizes electron probe x-ray microanalysis (EPMA) to measure the effects of various doses of cocaine, cocaethylene and alcohol as well as these drugs in combination on cellular elemental distribution in the leech ganglia model.

TECHNICAL PROGRESS AND RESULTS - Fiscal Year 1994:

Purpose: The purpose of this study is to investigate the contribution of the local anesthetic properties of cocaine to its toxic effects when given alone or in combination with alcohol. Since local anesthetic actions of cocaine lead to changes in cellular ion concentration through interaction with Na^+ and K^+ channels, we plan to measure these effects using electron probe x-ray microanalysis. Though most of the pharmacological actions of cocaine have been related to its sympathomimetic effects, the acute lethality from cocaine has been related to its anesthetic properties. It has also been shown that the combined use of cocaine and alcohol markedly increases the lethality of cocaine by eighteen fold. It has been postulated that the increase in toxicity is due

to the production of cocaethylene, a metabolite formed by the interaction of cocaine and alcohol. We propose, however, that the increased toxicity is brought about by the differential interference with ion exchange across membranes by alcohol and cocaine.

Approach: Initially we investigated the effects of cocaine and cocaethylene on the rate of uptake of Rb as a K marker into leech glial cells and neurons. The leech was chosen as an experimental model because its nervous system is relatively simple and has been extensively studied. Furthermore, it has large easily recognized neurons and distinctive glial cells making this preparation ideal for EPMA studies. In our initial studies we looked for changes in Rb uptake velocity into neurons and glial cells. Since leech glial cells are passive accumulators of K, if cocaine or cocaethylene affected the rate of uptake of Rb we could have concluded that these drugs might affect the neuron-glial homeostatic relationship by altering glial K buffering properties. These initial studies however failed to demonstrate any significant effect on Rb uptake, although we observed some curious and inconsistent effects on Na.

We then began studies to determine if elemental distribution was altered in neurons and glial cells in response to dose. Since any effects were likely to be over an extended time period, we chose to examine the effects of cocaine and cocaethylene dose on elemental distribution after 20 min. exposure to these drugs over the range of 10^{-6} , 10^{-5} , and 10^{-4}M . Using EPMA of frozen hydrated tissue sections we measured Na, K, Cl, S, P, Ca, Mg and H_2O content of leech neurons and glial cells.

Technical Progress and Results: As mentioned above, we did not observe an effect of cocaine or cocaethylene on Rb uptake in neurons or glial cells. Similarly we did not observe any change in K content in either

neurons or glial cells over the range of 1 μ M to 100 μ M. Cocaethylene also had no effect on K content. However, we did observe what appears to be a dose dependent effect of cocaethylene on glial Na and Cl but not on neurons (Fig. 1).

Over the dosage range studies, cocaine decreased glial Na in a dose dependent fashion; neuron Na remained unchanged except when exposed to the highest dose (10^{-4} M cocaine). Under those conditions neuron Na decreased nearly 30%. Interestingly glial Cl content was not affected by cocaine. Thus, the affects of cocaine on glial elemental content appear to be nearly entirely on Na. Neuron Cl on the other hand decreased in proportion to the Na decrease.

Neither cocaine or cocaethylene had any effect on Ca, Mg or P. Cocaine and 200 mM alcohol had no effect on glial Na or glial Cl. There did appear to be a dose related decrease in neuron Cl with the cocaine plus alcohol mixture. Alcohol alone had no effect on element content but significantly increased glial Na and Cl (Fig. 1). Water content and elemental content of leech neurons and glial cells, with the exception of Na and Cl (neuron) was not affected by cocaine.

Accomplishments: These data suggest that cocaethylene may have a pharmacological effect on glial cells. This is evidenced by the increase in glial Na and Cl with cocaethylene but not with cocaine. The effects of alcohol alone on glial Na and Cl may be a separate effect. Thus the combination of alcohol and cocaine may have opposing effects, but those effects are clearly different than cocaethylene effects. The ramifications of the elemental changes induced in glial cells by cocaine may be significant in affecting glial metabolism and their homeostatic function in the CNS. We believe that these results are the first evidence of a local anesthetic effect on glial cells. These cells have been largely overlooked because it

is widely assumed that local anesthetics work only on neurons (axons). The effect of cocaine on neuron Na may be an early toxic effect. No publications have, as yet, resulted from this work. However, we are encouraged by the interesting effects of cocaine on glial cells and we feel that this is new information which may have significance to both the action of local anesthetics in general and to the specific problem of cocaine toxicity.

LDRD FUNDING:

FY 1992	\$ 31,837
FY 1993	44,028
FY 1994	13,623

Figure 1

Calcification in Marine Alga: A Molecular Approach to a Major Pathway of Global Carbon Cycling

J. LaRoche

93-10

PROJECT DESCRIPTION:

The oceanic reservoir of inorganic carbon is 50 times that of the atmosphere and it exchanges with the latter on timescales of months to hundreds of years. At the most basic level, the internal cycling of carbon in the oceans is controlled by marine organisms through the production and transport of biogenic carbon in the form of 'soft' parts (organic tissue) and 'hard' parts (CaCO_3 shells). The production of hard parts of biocalcification reduces alkalinity and increases pCO_2 , opposing to some extent the effect of organic carbon production (decrease in pCO_2 but no effect on alkalinity). The coccolithophorid *Emiliania huxleyi* is the most abundant and most important organism producing CaCO_3 in the oceans and this is due to its ability to form dense blooms in both coastal and open oceans. Based on the restricted information available from geological records, the biology of coccolithophorids, and current scenarios for global warming, it is reasonable to envision positive feedback involving increased calcification that would tend to increase the accumulation of pCO_2 in the atmosphere and the global warming potential. It is proposed to study the process of calcification in *E. huxleyi* using molecular biological techniques to better understand which environmental factors regulate the expression of this process. Calcification is important, yet poorly studied and a combination of physiological, biochemical and molecular approaches will

provide a basic understanding of this process. The project is timely given the emphasis of funding agencies towards the study of globally important problems and the use of molecular techniques in environmental research.

TECHNICAL PROGRESS AND RESULTS - Fiscal Year 1994:

Purpose: The aim of this work is to identify the factors controlling calcification in the coccolithophorid *Emiliania huxleyi*. Previous work on the physiology of this species indicates that the ability to calcify has been irreversibly lost in some strains of *E. huxleyi*. This suggests that the ability to calcify in this species is in part controlled at the genetic level. *E. huxleyi* has a complex life cycle and can be observed as a naked motile cell, a naked or calcifying non-motile cell. Several environmental factors, such as nutrient concentrations or light, are also believed to affect calcification rates.

Approach: Two strains of *E. huxleyi* are used in the study of calcification. Strain CCMP 373 is a non-calcifying strain isolated in 1960. The calcifying strain, CCMP 374 is a recent isolate (1989) in which approximately 50% of the carbon fixed goes into the production of CaCO_3 . A comparison of the two strains at the physiological, biochemical and molecular level should give some indication of the metabolic pathways and enzymes involved in calcification.

Technical Progress and Results: The two *E. huxleyi* strains (CCMP 373 and CCMP 374) have been obtained from the Bigelow culture collection and are currently grown successfully in my laboratory. Culture conditions and media preparation have been optimized to ensure good yield of cells in 8 liter culture vessels. The cells are routinely counted using a light microscope containing light polarizing

filters that allow the enumeration of calcifying cells. The comparison of the two strains is in progress. Polyacrylamide gel electrophoresis has been used to compare total protein profiles, and western blots have confirmed the similarity between the major photosynthetic proteins, such as the light harvesting chlorophyll binding proteins, rubisco and D1, in the two *E. huxleyi* strains. DNA and RNA have been isolated from the two algal strains, and the randomly amplified polymorphic DNA (RAPD) fingerprinting technique based on the polymerase chain reaction is being developed to assess the similarity of the genome of these two strains. A cDNA library of the calcifying strain is under construction for differential screening in an attempt to isolate genes involved in the process of calcification. The protein profiles of the two strains have been compared using 2-dimensional (2-D) gel electrophoresis and the two strains appear to be quite different. Analysis of the 2-D gels using Bioimage software from Millipore (Dr. Dave Caron, WHOI) is expected. On the basis of these preliminary results, I have begun an attempt to generate a non-calcifying strain by growing CCMP374 in progressively higher nutrient conditions in order to make it lose its ability to calcify.

Experiments on nutrient limited cultures have shown that *E. huxleyi* does not grow in the presence of iron (>50 nM Fe). In addition, growth at pH 7.0 induced the swimming stage of the life cycle. Based on a series of preliminary experiments, I hypothesized that dissolved inorganic phosphate (but not organic phosphate) physiologically inhibits calcification by blocking an enzyme involved in the synthesis of a polysaccharide component of the organic matrix.

An analytical system to measure alkalinity in cultures of *E. huxleyi* was assembled and used to routinely measure calcification rates in cultures (Fig. 1). An experimental system was developed to look at the effect of various metabolic inhibitors and other environmental factors on calcification. *E. huxleyi* cells are decalcified by adding acid and bubbling with air. Upon reestablishment of the pH, the cells re-calcify before they resume cell division and growth (Fig. 2).

An immunofluorescence assay was developed using antibodies to cell surface antigens and tested in the laboratory. This antibody cross-reacted with all life stages of *E. huxleyi* but not with other species. This technique was used to demonstrate that *E. huxleyi* is very abundant in the area of Cape Hatteras where the Ocean Margins Program (OMP) will conduct a field study in 1996.

PAPERS/JOURNALS/PUBLICATIONS:

A presentation was made during the site review of the BNL contribution to OMP. A poster was presented at the annual OMP meeting in Woods Hole, Sept 17-19, 1994.

FOLLOW ON FUNDING:

A proposal was submitted to DOE's Ocean Margins Programs (OMP, OHER) and the work on calcification will be continued under an FWP entitled "Biogeochemical Cycles".

LDRD FUNDING:

FY 1993	\$ 58,370
FY 1994	60,515

Fig. 1. Increase in cell density (■ and dotted line) and decrease in alkalinity (● and solid line) with time in batch cultures of *E. huxleyi*.

Fig. 2. Decalcification (arrow) and recalcification of *E. huxleyi* as determined from the percent of lith-bearing cells in batch cultures (● and dotted line). Cell densities are represented by □ and solid lines.

Structure - Sensitive Properties of Advanced Permanent Magnet Materials: Experiment and Theory

*D. O. Welch and
L. Henderson Lewis*

93-11

PROJECT DESCRIPTION:

The goal of this program is to achieve an understanding of the properties and mechanisms which control the coercivity, remanent magnetization, and maximum energy product of advanced permanent magnet materials. These characteristic properties determine the practical usefulness of materials as permanent magnets in applications such as electric motors, sensors, and scientific instruments. These properties are extremely sensitive to the microstructure and crystal lattice defects on a scale of nanometers and are thus amenable to improvement by appropriate materials processing methods. This research program has both experimental and theoretical components and coexists symbiotically with the existing research programs on superconducting materials in the Materials Science Division of the Department of Applied Science, since there are many natural overlaps, especially the focus on the effect of crystal lattice defects on magnetic flux structures and properties, as well as a concern with the materials science of complex intermetallic compounds, in the research on both classes of materials.

This study, which is being done in collaboration with Carleton Fuerst et al., at the General Motors Research Laboratories, P. Panchanathan et al., Magnequench, and C.

Sellers et al., Idaho National Engineering Laboratory (INEL) focuses on the relationships between nanometer-scale defect structure and the structure-sensitive magnetic properties (coercivity, remanence, and magnetic viscosity) of advanced permanent magnet material, exemplified by the $R_2M_{14}X$ system, where B is a rare-earth element (Nd, Pr, ...), M is a transition metal (Fe, Co, ...), and X is a metalloid (B, C, ...).

TECHNICAL PROGRESS AND RESULTS - Fiscal Year 1994:

Purpose: The specific purpose of this work is to understand how various materials processing conditions lead to the development of crystal lattice defects organized in a nanometer-scale microstructure and how these microstructural elements control the structure-sensitive magnetic properties of an advanced permanent magnet material such as $Nd_2Fe_{14}B$. Understanding of this nature should lead to the development of methods for producing substantially improved permanent magnets for use in more efficient electric motors, for example. The various groups performing this research bring complementary strengths to the endeavor: the General Motors group was the discoverer of $Nd_2Fe_{14}B$ (independently and simultaneously with a group at Sumitomo in Japan) and has pioneered the use of rapid-solidification-based processing methods for this material; the Magnequench group has expertise in thermomechanical processing and is responsible for the commercial production of Nd-Fe-B magnets; the INEL group is collaborating with Magnequench to develop gas-atomization processing methods; and the BNL Materials Science group has substantial experience in microstructural and electromagnetic characterization of complex intermetallic compounds and superconductors, as well as in the theory of lattice defects and their properties in such materials. Another

purpose of this work is to establish a productive working relationship between these groups of industrial and national laboratory researchers.

Approach: Samples of $R_2M_{14}X$ (e.g. $Nd_2Fe_{14}B$, $Nd_2Co_{14}B$, $Nd_2Fe_{14}C$, Pr_2Nd_2B , etc.) and $PrCo$, are fabricated by various methods by Carleton Fuerst and his colleagues at GM, P. Panchanathan et al. at Magnequench, and C. Sellers et al. at INEL and supplied to the BNL group (L. Henderson Lewis, et al.) who then characterize the defect microstructure using advanced transmission electron microscope (TEM) techniques, as well as atomic force microscopy (AFM), and the magnetic properties are measured using superconducting quantum interference device (SQUID) magnetometry. Thermodynamic phase identification is done using tuneable-wavelength x-ray diffraction at the NSLS and the state of crystallographic texture is characterized using neutron scattering at the HFBR. Theoretical models of lattice defects and their interaction with the magnetic domain structure are developed by the BNL group (D. O. Welch, et al.).

Technical Progress and Results: The mechanism by which magnetic reversal occurs in the $RE_2Fe_{14}B$ die-upset magnets is an open question in the literature; the identification of the dominant mechanism is of great importance in the engineering of such magnets with enhanced coercivities. High resolution analytical electron microscopy and initial magnetization studies at elevated temperatures were performed at BNL on two die-upset rare-earth magnets with bulk compositions $Nd_{13.75}Fe_{80.25}B_6$ and $Pr_{13.75}Fe_{80.25}B_6$ fabricated by GM and Magnequench. In both compositions an amorphous intergranular phase was found which occurs primarily on those surfaces parallel to the tetragonal 2-14-1 phase c-axis.

The intergranular phase is enriched in iron relative to the bulk grain, with an average width of 8 - 12 Å in the Nd-based magnet and 15 - 20 Å in the Pr-based magnet. The initial magnetization curves and the dependence of the coercivity on maximum magnetizing field both increase linearly with a common slope and then saturate, consistent with nucleation-dominated behavior. These microstructural, microcompositional and initial magnetization data suggest that, contrary to the previous beliefs, the dominant reversal mechanism in these magnets is nucleation of reversed domains in the iron-rich intergranular region of reduced anisotropy. In fact, this previously undiscovered iron-rich phase appears to be ferromagnetic. Hysteresis loops were performed at 800 K on die-upset magnets with the above compositions. Each sample showed a clear hysteresis with coercivities between 34 and 40 Oe, average remanence $4\pi M_R$ of 6.8 G for the Nd-based sample and 10.3 G for the Pr-based sample. The ferromagnetic signals measured at high temperature in these magnets are attributed to the iron-rich grain boundary phase. The observations suggested an explanation for previously not-understood variations of the coercivity with the type of processing method used, and show the value of closely-coupled magnetic and microstructural characterization. Theoretical studies during this period featured work on the development of embedded-atom models of cohesion in transition-metal compounds. These models are used to estimate the properties of lattice defects and to help understand the location of alloying elements.

PAPERS/JOURNALS/PUBLICATIONS:

Fuerst, C. D., Brewer, E. G., Mishra, R. K., Zhu, Yimei, and Welch, D. O. Die-upset praseodymium-iron-boron-type magnets from melt-spun ribbons. *J. Appl. Phys.* 75, 4208-13 (1994).

Henderson Lewis, L., Zhu, Yimei, and Welch, D.O. Evidence for reversal by nucleation in RE-Fe-B die-upset magnets. J. Appl. Phys. (in press). Proc. of the 6th Joint Magnetism and Magnetic Materials-Intermag. Conf., Albuquerque, NM, June 20-23, 1994.

Henderson Lewis, L., Zhu, Yimei, and Welch, D.O. Ferromagnetic grain boundary signature in die-upset RE-Fe-B magnets. Acta Met. Mag. (in press). Proc. of the Acta Metallurgica Meeting on Novel Magnetic Structures and Properties, Sante Fe, NM, June 24-25, 1994.

FOLLOW-ON FUNDING:

This program was one of the three winners of the DOE Basic Energy Sciences/Division of Materials Sciences New Initiative Competition for FY 1996. [Partial transitional funding will be available in FY 1995, with full funding to commence in FY 1996.]

LDRD FUNDING:

FY 1993	\$ 99,186
FY 1994	105,710

A High Flux Neutron Source

J. Powell, H. Ludewig
and M. Todosow

93-20

PROJECT DESCRIPTION:

Particle Bed Reactor (PBR) technology is being investigated for application to the design of a high power density spallation neutron source. Data from the Space Nuclear Thermal Propulsion (SNTTP) program, and other sources is being applied to this design.

The baseline parameters of such a source are that it operate in a pulsed mode (10Hz-50Hz), and with a proton beam power of 5MW. In order to ensure the most intense source possible for a given target material, it must occupy the smallest volume consistent with efficient heat removal, overall neutron production, and availability of neutrons for experiments. These requirements can best be satisfied by a particulate bed target arrangement, if the target is to remain solid, for the following two reasons.

- 1) Particle beds have the highest heat transfer area per unit volume of any practical target arrangement, and
- 2) The cyclic stresses due to the pulsed nature of the source will be minimized in the case of a particulate bed compared to any other geometric arrangement, thus minimizing fatigue failures.

The above two properties of particle beds have been confirmed experimentally in

the SNTTP program, and these basic results will be used in the design of a spallation neutron source. Targets operating at a maximum power density of 10 MW/L are well within the capability of such a target design.

TECHNICAL PROGRESS AND RESULTS- Fiscal Year 1994

Purpose: A feasibility study of a compact high power density target for a spallation neutron source was undertaken. The target arrangement consists primarily of heavy metal, with appropriate cooling passages. A high intensity proton beam of intermediate energy is directed at the target; where it interacts with the heavy metal nuclei. The subsequent spallation reactions produce several neutrons per proton resulting in an intense neutron source. The proton beam is assumed to have an energy of 5 MW, and to be cyclic with a repetition rate of 10Hz - 60Hz.

The study was divided into two broad sections. First, an analysis of preliminary target designs was undertaken to ensure the overall feasibility of the concepts involved in the design and eventual construction of such a high power density target. Second, two proposed target designs, based on the first set of analyses, are investigated in more detail. Special care is taken to ensure that the neutron fluxes in the moderator are at the desired level, no material compatibility problems exist, and the target is able to operate in a reliable and safe manner.

Several target materials, coolant types, and target arrangements are investigated. Followed by a detailed study of a single target material and geometric arrangement. Several structural material choices are investigated with the aim of minimizing the effects of

structural heating, and associated thermally induced stresses.

Approach: The major performance related objective for the BNL Pulsed Spallation Neutron Source (PSNS) target and moderator system is to provide the maximum flux possible for experiments. In addition, there should be an ability to tailor the flux spectra and time-structure characteristics to allow optimal utilization of the spallation neutrons. These objectives impact the target geometry and materials, the selection of moderator/reflector, and the arrangement of the beam lines and local moderators.

The design of the target and moderator system for the PSNS was based on a series of trade studies that were performed to evaluate the impact of a number of possible design options on engineering feasibility and expected performance. In addition to evaluating the neutron production and resultant fluxes, the design efforts addressed engineering details, including energy deposition and heat removal from the target and related components, safety, handling, shielding, neutron utilization, etc. The parameters considered in the design studies were based on examination of current and proposed spallation targets, and extensive experience at BNL in the design of accelerator-driven targets and reactor systems obtained in connection with the Accelerator Production of Tritium (APT), Space Nuclear Thermal Propulsion (SNTTP), and other programs.

Some of the design options considered were:
Proton Energy - 1 to 10 GeV

Target Material - Tungsten, Uranium, Lead

Structural Material - Stainless Steel or Aluminum

Coolant- Sodium, Lead, Helium, Heavy Water

Geometry - Rods, Particles

Technical Progress and Results: The baseline BNL PSNS target is shown schematically in Figure 1. It consists of three target regions, separated by two void zones known as "flux traps." Slab moderators are positioned opposite the flux traps, and above and below the "solid" target sections; these latter moderators are known as "wing moderators." Each moderator block has associated with it one or two beams. The energy spectrum of the emitted source neutrons is modified by the slab moderators, and the neutrons then travel through the beam tubes to the various experimental end-users. Liquid hydrogen, deuterium and methane, and light water are candidates for the beam-tube slab moderators. The entire target, moderator blocks and beam tubes is embedded in a "moderator/reflector" region. In the present studies the surrounding moderator has been assumed to be liquid water, but in principle could be any moderator material (e.g., beryllium, plastic, etc. that can survive the temperature and radiation environment). This region could also contain heavy materials such as nickel, and thereby serve as part of the reflector/shield structure. While water has been assumed for the present configuration because it eases target and beam tube handling, inspection, and repair, the choice is still open. In some situations it may be desirable to de-couple the flux in the beam tube from that in the surrounding regions, thereby changing the time structure of the neutron pulses available to experimenters. In these studies, decoupling was achieved with a thin cadmium layer on selected beam tubes and associated slab moderator blocks.

A distinguishing characteristic of the target regions in Fig. 1 is that each represents a particle bed of tungsten spheres 1 - 2 mm in diameter. The spheres (particles) are retained between two porous walls (frits), and are cooled

directly by heavy water which flows from an inlet plenum into the particle bed through the "cold frit." After passing through the bed, the coolant exits through the "hot frit" into an outlet plenum and then flows to a heat exchanger.

The particle bed approach was selected for the target because the performance objectives discussed above, along with the results of trade-studies performed at BNL and elsewhere suggest that the "optimum" target should be a balance between sufficient size to maximize the neutron production per proton, and a configuration that maximizes the leakage of the neutrons out of the target to where they can be used productively. This implies a compact target(s), which in combination with a relatively high proton beam-power results in high power densities. The excellent heat transfer characteristics of a particle bed configuration (resulting from a very high surface-to-volume ratio), make it particularly well suited for these conditions.

The parameters for the baseline target are given in the following table:

Table 1

Parameters for Target/Moderator System
Description - Split Particle Bed with 2-Flux Traps
Tungsten Spheres ~1 to 2 mm OD
Stainless Steel Frits
External Neutron Multiplier (lead or beryllium)
Heavy Water coolant
Thermal-hydraulic Operating Conditions
Coolant Operating Pressure (4-6Bar)
Coolant Outlet Temperature (80°C)
Particle Surface Temperature (<116°C)
Coolant Saturation Temperature (144°C)
Energy-Integrated Neutron Flux @ 5MW
Maximum Flux on Surface of target
~7x10 ¹⁴ n/cm ² -sec

The above results show that the expected operating temperatures and pressures are modest (as are the required coolant flow rates), with adequate margins to coolant boiling and tungsten melting ($T_{mp} = 3410^{\circ}\text{C}$).

The major safety issues associated with the target involve material behavior (oxidation, fatigue and mechanical shock), and heat removal in the event of an upset.

The targets are expected to operate at a frequency of 10 -50 Hz; therefore the particles, and to a lesser extent the frits will be subjected to a varying temperature environment, which implies an undulating stress. In addition, the accelerator is able to produce its full beam power almost instantaneously, thus the thermal stress will be applied extremely rapidly. The concerns associated with these issues are fatigue failure of the target after a short operating time, and intolerable shock loads. These issues are both dependent on the temperature gradient generated across the components. Although the particles operate at a high power density, this effect is not expected to be serious since the particles are small, and tungsten is a good conductor; the resulting temperature rise is less than one degree across the particle. The resulting thermal stress is also extremely small (approximately 150 psi) and well within the limitations of tungsten. Furthermore, the rate and frequency at which the stress is applied should not cause any fatigue or shock load problems.

The other material issue associated with tungsten is the possibility of oxidation in the event of an over-temperature transient. Despite the high melting temperature of

tungsten it is prone to attack by oxygen (tungsten oxide sublimates at 800 - 1000 K). This issue has not been quantified as yet; however, control of the free oxygen content of the coolant through a recombiner may be required.

The choice of tungsten and stainless steel as the target materials, and the low operating temperatures (approximately 400K max.) result in large thermal margins. Thus, these materials are very tolerant to under-cooling transients, such as pipe breaks or leaks, and should allow sufficient time for the back-up cooling system to operate. Several back-up systems are being considered. The most likely candidate is a system consisting of a large pressurized source of back-up cooling water. This system would be separated from the primary system by check valves, and automatically activated in the event of a drop in primary system pressure. Analyses to verify this design are still required. It should be noted that the efficient heat removal possible with a particle bed will also enhance the removal of post-accident heat from a target. Finally, light water can be used to provide additional back-up cooling since there are no criticality concerns.

LDRD FUNDING:

FY 1993	\$38,895
FY 1994	44,306

Spallation Target and Moderator Configuration

Schematic representation of the three-section spallation target. High energy neutrons generated in the target sections will be scattered into six angled wing moderators and four flux-trap moderators supplying lower energy neutrons to sixteen beam lines

Figure 1

Genetics of Drug Addiction

C. Ashby

93-22

PROJECT DESCRIPTION:

The purpose of the current proposal was to use the techniques of single cell recording and microiontophoresis to examine the function of pre- and postsynaptic dopamine (DA) receptors in the mesocorticolimbic system of anesthetized male Fischer 344 (F344) and Lewis rats. Specifically, the specific experiments to be conducted for this proposal were as follows: 1) determination of the number, as well as the firing rate and firing pattern, of spontaneously active A9 and A10 DA cells; 2) examination of the response of spontaneously active A9 and A10 DA cells to intravenously administered cocaine (0.25-8 mg/kg), 7-OH-DPAT (D3 agonist, 0.5-8 µg/kg), (+)-apomorphine (D2 autoreceptor agonist, 1-32 µg/kg) and methamphetamine (DA releaser, 0.1-3.2 mg/kg); 3) examine the response of spontaneously active or L-glutamate-activated neurons in the nucleus accumbens (NAc), striatum and medial prefrontal cortex (mPFC) to the iontophoresis (2.5-80 nanoamperes) of DA, GABA, cocaine and the D1 and D2 receptor agonists SKF 81297 and quinpirole, respectively; 4); the response of mPFC and NAc cells to DA, SKF 81297, and quinpirole will be ascertained after the acute and chronic administration of cocaine and 6) use the technique of in vivo microdialysis in freely moving rats to measure extracellular levels of DA in the NAc.

I would like to point out that I have slightly modified the original statement of work. Originally, I stated that I would place a stimulating electrode in the A10 area, isolate cells in NAc and mPFC with a glass

micropipette and quantitate the effects on neuronal firing rate and pattern. However, I decided to omit this as recent data indicate that the stimulation of the VTA region produces the release of DA, 5-HT and other neurotransmitters in the NAc and mPFC, thereby making it difficult to interpret the results. Consequently, I decided to use the technique of microdialysis, which allows the exact measurement of extracellular levels of neurotransmitters in the brain of awake, freely moving animals. Thus, the interpretation of the results is more straightforward. In addition, the experiments examining the response of 5-HT neurons in each strain to the i.v. administration of 8-OH-DPAT were omitted. Instead, experiments determined the effect of (±)-7-OH-DPAT on midbrain DA neurons in F344 and Lewis rats were conducted. These experiments will provide important data about the DA system.

TECHNICAL PROGRESS AND RESULTS - Fiscal Year 1994:

Purpose: Previously, it has been shown in animals and humans that there are significant differences between individuals regarding vulnerability to self-administer addicting drugs. In addition, numerous studies indicate that this vulnerability has a strong genetic component. Research over the past three decades has demonstrated that the rewarding or reinforcing effects of addicting drugs is mediated by dopaminergic neurons in the mesocorticolimbic areas. Consequently, one might hypothesize that the differences between individuals regarding drug abuse may be related to differences in the function of DA neurons in the mesocorticolimbic system, which may be genetically controlled. For obvious ethical and technological reasons, this hypothesis can't be readily tested using human subjects.

However, recent studies have shown that there are marked differences between two strains of rats, F344 and Lewis rats, with regards to their propensity to self-administer drugs of abuse and their behavioral response to various drugs. Furthermore, these strains of rats are genetically distinct and have been inbred for over 80 generations. Therefore, each individual within a strain is virtually identical to the other individuals in terms of its allelic identity.

Thus, based on the above information, I decided to use the techniques of in vivo extracellular single cell recording and iontophoresis and microdialysis to examine the responsiveness of pre- and postsynaptic DA receptors in the mesocorticolimbic system. In addition, as a control, the responsiveness of DA receptors in the nigrostriatal system, which does not play a significant role in mediating the rewarding effects of drugs of abuse was also examined.

Technical Progress and Results: **A. The determination of the number of spontaneously active DA neurons in the A9 and A10 areas of the F344 and Lewis rats:** The results obtained from these studies are shown in Table 1. Overall, the Lewis rats had significantly fewer spontaneously active A9 and A10 DA neurons compared to F344 rats. Currently, we are pursuing research to gain insight into this phenomenon. In addition, at this time, we do not know if there is a correlation between our findings and the difference in the strains to self-administer drugs of abuse.

B. The measurement of parameters indicative of the firing pattern of A9 and A10 DA neurons in F344 and Lewis rats: A summary of the results obtained regarding the firing pattern of A9 and A10 DA neurons is presented in Table 2. It should be noted that

A10 DA neurons are generally more irregular (i.e., "bursty") with regard to their firing pattern compared to A9 DA neurons. This difference between A9 and A10 DA cells has been reported for other rat strains. The A10 DA neurons in the Lewis rats showed a significantly greater increase compared to the F344 Lewis with regards to spikes/burst, total number of bursts and % of DA neurons exhibiting a burst firing pattern. In addition, there were a greater % of A9 DA neurons in the Lewis rat exhibiting a burst firing pattern compared to F344 rats.

C. The effect of intravenously administer cocaine and methamphetamine on the basal firing rate of A9 and A10 DA neurons in F344 and Lewis rats: The results of these studies are presented in Tables 3 and 4. Our results indicated that there was no significant in the ED₅₀ (dose of cocaine required to suppress DA neuronal firing by 50%) values for cocaine in the A10 area between F344 and Lewis rats. However, in the A9 area, cocaine was about 3 times more potent in suppressing the basal firing rate of A9 DA neurons in the F344 compared to the Lewis rats. In addition, cocaine was more potent in suppressing the basal firing rate of A10 DA neurons compared to A9 DA neurons in the Lewis rats. In contrast, there was no difference between the two strains in the ED₅₀ values for methamphetamine.

D. The effect of the DA autoreceptor agonists (+)-apomorphine and (±)-7-hydroxy-di-n-propylaminotetralin (7-OH-DPAT) on the basal firing rate of spontaneously active A9 and A10 DA cells in the F344 and Lewis rats: The data from these experiments are summarized in Tables 5 and 6. There was no significant difference in the ED₅₀ values obtained for (+)-apomorphine between the F344 and Lewis rats. In contrast, (±)-7-OH-DPAT was significantly more potent

in suppressing the basal firing rate of A10 DA neurons in the Lewis compared to the F344 rats.

E. The determination of the extracellular basal levels of DA and its two major metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in the nucleus accumbens and the effect of cocaine administration: Overall, there was no significant difference between the strains in the extracellular basal levels of DA in the nucleus accumbens (Figure 1). In contrast, basal levels of HVA and DOPAC (two major metabolites of DA) in the nucleus accumbens were significantly lower in the Lewis compared to the F344 rats. There was no significant difference between the strains in the extracellular levels of DA, HVA or DOPAC measured after the administration of 3, 10 or 30 mg/kg i.p. of cocaine (Figure 2). However, after a second injection of 30 mg/kg i.p. of cocaine, the absolute increase as well as the duration of increase in DA levels in the nucleus accumbens of the Lewis rats was significantly greater than that of F344 rats.

PAPERS/JOURNALS/PUBLICATIONS:

Strecker, R.E., Eberle, W.E. and Ashby, Jr., C.R. (1993) Cocaine-induced changes in extracellular dopamine (DA) levels in the nucleus accumbens of Fischer and Lewis rats. *Soc. Neurosci. Abstr.* 19: 1825 (Abstract no. 745.2).

Strecker, R.E., Eberle, W.E. and Ashby, Jr., C.R. (1994) The measurement of basal and cocaine-induced extracellular levels of DA, HVA and DOPAC in Fischer (F344) and Lewis rats: an in vivo microdialysis study. Submitted to *Life Sciences*.

Ashby, Jr., C.R., Minabe, Y. and Emori, K. (1994) The determination of the number of spontaneously active DA neurons and their firing pattern in the A9 and A10 areas of the Fischer (F344) and Lewis rats: an in vivo electrophysiological study. Submitted to *Life Sciences*.

Ashby, Jr., C.R., Minabe, Y., Emori, K. and Toor, A. (1994) The effect of various direct and indirect DA receptor agonists on the basal firing rate of A9 and A10 DA neurons in Fischer (F344) and Lewis rats: an electrophysiological study. Manuscript in preparation.

FOLLOW-ON FUNDING:

Based on preliminary data obtained from this proposal, a grant will be submitted to NIDA on March 1, 1995.

LDRD FUNDING:

FY 1993	\$ 68,023
FY 1994	87,391

Note: This project involves vertebrate animals.

Table 1: A summary of the electrophysiological experiments determining the number of spontaneously active DA neurons in the A9 and A10 areas of F344 and Lewis rats.

Species	Brain Area	
	A9	A10
F344	$0.89 \pm 0.06^1 (17)^2$	$1.47 \pm 0.07 (16)$
Lewis	$0.28 \pm 0.04 (17)^*$	$0.51 \pm 0.04 (16)^{**}$

¹Each value represent the mean number of spontaneously active DA neurons encountered per electrode track $\pm$ S.E.M.

²The numbers in parentheses represent the number of animals examined.

*Significantly less than F344 rats, $t = 8.61$, $df = 31$, $p < 0.01$, Student's t test.

**Significantly less than F344 rats, $t = 11.2$, $df = 31$, $p < 0.01$, Student's t test.

Table 2: A summary of the burst firing analysis for DA neurons in the A9 and A10 areas of F344 and Lewis rats.

Parameter assessed	A9-Lewis	A9-F344	A10-Lewis	A10-F344
spikes/burst	2.23 ± 0.22^1	2.16 ± 0.18	2.77 ± 0.16	$1.99 \pm 0.17^{**}$
% events in burst	7.26 ± 1.48	8.03 ± 2.65	22.8 ± 3.3	$9.5 \pm 2.2^{**}$
Interspike interval	260 ± 16	288 ± 18	255 ± 15	286 ± 22
total no. of bursts	14 ± 2.9	17 ± 7.3	61 ± 7.2	$17.9 \pm 4.9^{**}$
coefficient of variation	41.7 ± 4.8	35.6 ± 3.8	63.4 ± 3.1	52.9 ± 4.9
% cells bursting	29.3%	14.3%*	75%	33%*
basal firing rate (Hz)	3.8 ± 0.25	3.5 ± 0.22	3.9 ± 0.23	3.5 ± 0.27

¹Each value, except that of % cells bursting, represents the mean $\pm$ S.E.M. For each strain, 46-55 neurons were examined in the A9 and A10 area.

*Significantly less than that of Lewis rats, chi-square, $p < 0.05$.

**Significantly less than that of Lewis rats, Student's t test, $p < 0.01$.

Table 3: The effect of cocaine on the basal firing rate of A9 and A10 DA neurons in F344 and Lewis rates.

	ED ₅₀ of cocaine (mg/kg)	
	A9	A10
F344	1.8 ± 0.4 ¹ (11) ²	1.2 ± 0.16 (12)
Lewis	5.6 ± 1.10 (10)*	2.1 ± 0.5 (12)

¹ Each value represents the Mean ED₅₀ value ± S.E.M.

² The values in parentheses represent the number of DA neurons examined.

* Significantly greater than F344, p<0.01, Student's t test.

Table 4: The effect of methamphetamine (MAP) on the basal firing rate of A9 and A10 DA neurons in F344 and Lewis Rats.

	ED ₅₀ value of MAP (mg/kg)	
	A9	A10
F344	1.09 ± 0.1 ¹ (13)	0.88 ± 0.11 (13)
Lewis	1.38 ± 0.21 (12)	0.98 ± 0.11 (14)

¹Each value represents the mean ± S.E.M.

²The values in parentheses represents the number of animals examined.

Table 5: The effect of (+)-apomorphine on the basal firing rate of A9 and A10 DA neurons in F344 and Lewis rats.

ED ₅₀ of (+)-APO (μg/kg)		
	A9	A10
F344	7.4 ± 1.1 ¹ (9) ²	6.8 ± 1 (10)
Lewis	10.9 ± 1.5 (9)	6.6 ± 0.8 (11)

¹ Each value represents the mean ED₅₀ value ± S.E.M.

² The values in parentheses represent the number of DA neurons examined.

Table 6: The effect of (±)-7-OH-DPAT on the basal firing rate of A9 and A10 DA neurons in F344 and Lewis rats.

ED ₅₀ value of 7-OH-DPAT (μg/kg)		
	A9	A10
F344	5.6 ± 0.9 ¹ (7) ²	7.7 ± 0.9 (7)
Lewis	7.0 ± 1.1 (7)	2.5 ± 0.6 (7)*

¹ Each value represents the mean ED₅₀ value ± S.E.M.

² The values in parentheses represent the number of animals examined.

* Significantly less than F344, p<0.01, Student's t test.

Figure 1: Extracellular basal levels of dopamine, DA, HVA and DOPAC in the nucleus accumbens.

Figure 2: Extracellular levels of DA, HVA and DOPAC measured after administration of 3, 10 and 30 mg/kg i.p. of cocaine.

Development of Microdialysis Technology

Stephen L. Dewey, Ph.D.

93-24

PROJECT DESCRIPTION:

In vivo microdialysis in freely moving rats is an important neurophysiologic methodology that has been applied to the study of neurotransmitter interactions in the corpus striatum of adult male rats. While this methodology is useful for studies designed to examine the responsiveness of a given neurotransmitter system to a pharmacologic challenge, it also provides invaluable information concerning drug pharmacokinetics and pharmacodynamics. These data have been directly applied to the design and subsequent interpretation of positron emission tomography (PET) studies in the primate and human brain.

Specifically, studies have focused on the ability of serotonin, cocaine, methylphenidate, the opiates, GABA, acetylcholine, and amphetamine to modulate striatal dopamine. Data from these studies demonstrate that several different neurotransmitter systems maintain the ability to modulate dopamine release in a specific, inhibitory or excitatory, manner. The importance of this data goes beyond the assessment of basic neurophysiologic mechanisms associated with neurotransmitter release and provides insight into alternate therapeutic treatment strategies that may be effective for the proper management of many 'neurotransmitter-specific' neurologic diseases. For example, these studies demonstrated that the serotonin (5-HT₂)

receptor antagonist, altanserin, is capable of increasing synaptic acetylcholine concentrations as evidenced by a decrease in labeled benztropine binding. This may be important in the treatment of neurologic disorders associated with cholinergic hypoactivity (i.e., Alzheimer's disease). Finally, *in vivo* microdialysis studies demonstrate that our continued use of a general anesthetic agent for all primate PET studies does not alter the type of interaction that occurs between two functionally-linked neurotransmitter systems.

TECHNICAL PROGRESS AND RESULTS - Fiscal Year 1994

Purpose: *In vivo* microdialysis is a relatively new neurophysiologic technique that offers many advantages over other more indirect measures of neurochemical changes resulting from pharmacologic manipulation. With the surrounding neuropil intact, studies can be conducted that address questions of drug delivery and dose, pharmacologic responsiveness, receptor selectivity, and experimental design. When performed in conjunction with ongoing PET studies in primates, human volunteers and patients, the technique offers a unique ability to directly answer many questions that PET data can only infer.

Approach: The microdialysis technique requires expertise in high pressure liquid chromatography (HPLC) with electrochemical detection and stereotaxic surgery. The microdialysis probe is inserted into a surgically placed guide cannulae. The perfusion medium (artificial cerebrospinal fluid) is pumped through the probe at precisely controlled flow rates.

Samples are then auto-matically injected onto an HPLC and analyzed.

Technical Progress and Results: Early in fiscal 1994, we added a second automated HPLC and freely moving system to our existing microdialysis facility. This resulted in a marked increase in productivity and experimental throughput. All samples are now analyzed on-line which offers many advantages over our previous methods of injecting samples overnight and unattended. This year studies have been completed that were designed to investigate alternate treatment strategies for various pathologic conditions. By investigating the ability of one neurotransmitter to modulate the release of another, we can begin to target drugs towards indirectly modulating other functionally-linked systems.

For example, we examined the effect of altanserin and citalopram on the extracellular release of DA with *in vivo* microdialysis techniques. Altanserin administration increased extracellular DA levels within 20 minutes following administration. DA levels returned to near baseline values 80 minutes following administration. Citalopram, however, caused a gradual decrease in extracellular DA levels that were less than 40 % of baseline values 250 minutes following administration (20 mg/kg). Together, these findings are consistent with our primate PET studies that indicate that at between 40-60 minutes following altanserin administration, ^{11}C -raclopride binding is decreased while extracellular DA levels are elevated and that at 180 mins following citalopram administration, ^{11}C -raclopride binding is increased while extracellular concentrations are decreased.

In vivo microdialysis studies have been performed in freely moving rats using the identical experimental protocol performed for a separate set of primate PET studies (haloperidol (1.0 mg/kg) and clozapine (20 mg/kg) with the two exceptions that doses were modified for rats and the route of administration was intraperitoneal injections rather than PO; treatment duration and washout was identical). Rats were challenged with amphetamine, GVG, and citalopram in order to directly examine the responsiveness of DA, GABA, and 5-HT, respectively. These experiments examined extracellular DA, GABA, and 5-HT directly with high pressure liquid chromatography (HPLC) and electrochemical detection. These studies directly measured the responsiveness of the particular neuro-transmitter system to a direct challenge.

In a series of *in vivo* microdialysis studies, 6 rats were exposed to haloperidol for 1 week and washed out for one week while a second group of 6 rats was exposed to haloperidol for 5 weeks and washed out for one week. Following the establishment of a DA baseline in the dialysate, rats were injected with amphetamine (2.5 mg/kg, ip). In the saline treated control animals, DA levels were elevated 650% above baseline following amphetamine administration. In rats exposed to haloperidol for 1 week and washed out for one week, DA concentrations were elevated 900% above baseline. In rats exposed to haloperidol for 5 weeks and washed out for one week, DA concentrations were elevated 950% above baseline. These changes in the responsiveness of the DA system to an amphetamine challenge were significantly different ($p < 0.01$) from

the saline treated controls. These data suggest that acute and chronic haloperidol exposure results in a hyper-responsive striatal DA system and that this hyper-responsiveness occurs following a 1 week exposure and persists in animals exposed for 5 weeks. In a series of *in vivo* microdialysis studies, 6 rats were exposed to clozapine for 1 week and washed out for one week while a second group of 6 rats was exposed to clozapine for 5 weeks and washed out for one week. Following the establishment of a DA baseline in the dialysate, rats were injected with amphetamine (2.5 mg/kg, ip). In the saline treated control animals, DA levels were elevated 650% above baseline following amphetamine administration. In rats exposed to haloperidol for 1 week and washed out for one week, DA concentrations were elevated 1000% above baseline. In rats exposed to haloperidol for 5 weeks and washed out for one week, DA concentrations were elevated 1150% above baseline. These changes in the responsiveness of the DA system to an amphetamine challenge were significantly different ($p < 0.01$) from the saline treated controls. These data suggest that acute and chronic clozapine exposure results in a hyper-responsive striatal DA system and that this hyper-responsiveness occurs following a 1 week exposure and persists in animals exposed for 5 weeks.

Taken with the acute and chronic primate PET studies, the microdialysis data support the following interpretations of the underlying mechanisms responsible for the changes observed in the labeled raclopride studies. First, in the primate studies it would appear that the alteration in the ability of GABA to modulate striatal DA in

the acute or residual study probably occurs through the inability of GVG to raise GABA levels, or the inability of the GABAergic neuron to release GABA, as the microdialysis data suggests that the DA system is hyper-responsive following haloperidol administration. Second, the potentiated responsiveness of the DA system measured with *in vivo* microdialysis is clearly evident. When challenged with citalopram the normal response was a 45.9 % increase in labeled raclopride binding. Following 1 week exposure to haloperidol, citalopram produced a 68.9 % increase in labeled raclopride. This suggests that the hyper-responsiveness of the DA system maybe the underlying mechanism responsible for the measured effect. Support of these explanations requires completing *in vivo* micro-dialysis studies using citalopram and GVG and measuring the response of 5-HT and GABA, respectively. While direct comparisons between the primate PET studies and the *in vivo* microdialysis studies in rats must be made with caution, the combined use of PET and microdialysis provides a unique approach to understanding the consequences of acute and chronic neuroleptic treatment on neuro-transmitter interactions. Not only does the microdialysis data aid in our interpretation of the PET findings, it provides further evidence for the notion that centrally acting drugs effect different neurotransmitter systems at different times following their administration. For example, these very preliminary data suggest that the DA and GABA systems are affected 1 week following haloperidol administration while the 5-HT system may not be effected at all. However, with continued exposure to haloperidol the 5-HT system becomes

involved as evidenced by the attenuated response to a citalopram challenge. In a separate group of studies, animals were pretreated with GVG (at doses of 100 mg/kg and 300 mg/kg) and challenged with cocaine. Our previous dialysis data demonstrated that a dose of 20 mg/kg of cocaine increased striatal dopamine by nearly 250%. GVG pre-treatment, however, greatly attenuated the responsiveness of the dopaminergic system to a similar dose of cocaine (45%

increase). This is indicated in the figure where a 100 mg/kg dose of GVG does not attenuate cocaine-induced striatal dopamine release but a dose of 300 mg/kg of GVG significantly reduces dopamine release subsequent to a cocaine challenge.

These data suggest that GVG administration may provide an alternate treatment strategy for cocaine abuse. This is a unique finding and provides support for our working hypothesis that it may be more prudent to pharmacologically target neurotransmitter systems other than those suspected of underlying a particular disorder.

Taken together, these *in vivo* microdialysis studies demonstrate the utility of this technique for aiding and designing

more complicated neuro-imaging studies (PET and single photon emission computed tomography, SPECT). Furthermore, this technique provides complimentary data that aids in the interpretation of our imaging studies.

Finally, through the combined use of PET and *in vivo* microdialysis, we can begin to better understand many basic neurophysiologic processes that underlie the fundamental mechanisms used by the brain to respond to changes in our internal or external environment. Through this multimethodologic approach, it is our hope that we can design more rational treatment strategies for many debilitating neurologic disorders.

PAPERS/JOURNALS/PUBLICATIONS:

Dewey, SL, Smith, GS, Logan, J, Alexoff, D, Ding, Y-S, King PT, Pappas N, Brodie, JD, Ashby CR: Serotonergic modulation of striatal dopamine measured with positron emission tomography (PET) and *in vivo* microdialysis. *J. Neuroscience* (In Press, 1994).

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Dewey, SL, Smith, GS, Alexoff, DL, Ding, Y-S, Fowler, JS, Volkow, ND, Wolf, AP. Changes in dopamine with PET and microdialysis. *American Psychiatric Association* (In press, 1994).

Dewey, SL, Tan, P, Smith, GS, King, P, Pappas, N, MacGregor, R, Ding, Y-S, Shea, C, Alexoff, D, Martin, T, Gatley, SJ,

Fowler, JS, Wolf, AP. PET and in vivo microdialysis studies of SR 46349B, a new and selective 5-HT₂ antagonist. Soc. Neurosci. Abstr., 20: 1552, 1994.

Kasten, MR, Clinton, MA, Ulrich, AM, Alexoff, DL, Smith, GS, Ashby, CR, Dewey, SL. Effects of haloperidol and clozapine on amphetamine induced dopamine release measured with in vivo microdialysis in freely moving rats. Soc. Neurosci. Abstr., 20: 1560, 1994.

FOLLOW-ON FUNDING:

PO1 NS-15638 "Positron emitters and PETT in metabolism and neurology" Joanna Fowler, Principle Investigator, NINDS renewal

application, \$6,559,119, "Dopamine modulation through its interactions with other neurotransmitters" S.L. Dewey, Principle Investigator.

MH 49165 "PET investigations of neurotransmitter interactions" S.L. Dewey, Principle Investigator, NIMH, renewal application, \$950,000.

Established Investigator Award, National Alliance for Research on Schizophrenia and Depression (NARSAD), \$68,512.

LDRD FUNDING:

FY 1993	\$ 50,150
FY 1994	61,526

Note: This project involves vertebrate animals

Investigation of the Utility of Maximum-Entropy Methods for the Analysis of Powder Diffraction Data

D. E. Cox

93-25

PROJECT DESCRIPTION:

The application of the maximum-entropy (MaxEnt) method to the detailed analysis of powder diffraction data is being explored. MaxEnt will first be applied to high-resolution synchrotron x-ray data as a means to locate light atoms in inorganic compounds containing heavier ones, which is a very common problem in structural studies of polycrystalline inorganic materials such as complex high T_c oxides and heavy metal oxide catalysts. Other goals are to use MaxEnt to provide details of the bonding electron density distribution, and for the abinitio solution of unknown structures. Experimental work will be aimed at developing optimum strategies for the collection of suitably accurate powder data at beam-line X7A at the NSLS in order to get the best possible structural information.

TECHNICAL PROGRESS AND RESULTS - Fiscal Year 1994:

Purpose: The development of high-resolution synchrotron x-ray powder diffraction techniques at beam-line X7A at the NSLS over the past few years has demonstrated the utility of such data for the detailed structural characterization of a wide range of inorganic materials, including many high T_c systems and complex oxides of interest in catalysis. These studies have generated growing interest in the application of powder data to the abinitio solution of unknown crystal structures,

traditionally a technique requiring suitably-sized single crystals. However, because of limitations inherent to powder compared to single crystal data, such as poorer counting statistics, peak overlap and inferior peak-to-background discrimination, the information contained in a set of structure factors is generally less complete and of lower accuracy. Fourier maps derived from these data tend to be quite noisy, and information about light atoms, thermal fluctuations or bonding effects may be obscured.

Most generally, MaxEnt is the nonparametric method of choice to retrieve information from sparse and noisy data sets. The great enhancement possible with the use of MaxEnt stems from its ability to reduce truncation and noise effects by orders of magnitude. The method works best when the data relate linearly to the physical information sought. Some recent papers in the literature have demonstrated that MaxEnt can be a very powerful tool in the analysis of powder data, for it is the least-biased with respect to missing data and may reveal details not seen on Fourier maps. The purpose of the present work is to apply the MaxEnt method to high-resolution synchrotron data collected at X7A and assess to what extent it can enhance the structural information about the complicated systems typically encountered in materials research.

Approach: There are two difficulties to be faced in structure determination from powder diffraction data; the general problem of non-linear phases and the specific problem of overlapping of Bragg peaks. The use of MaxEnt to solve the former is a topic of much current interest, although phasing the data can frequently be accomplished with standard techniques.

In the first stage of the project, MaxEnt will be applied to the analysis of data

collected from selected reference materials as a benchmark test of the level of detail that can be extracted for simple systems. Particular attention will be given to the minimization of systematic errors in the data collection process, and to a realistic evaluation of the esd's. Subsequent applications will be to more complex heavy metal oxides containing Bi and Mo, for example, to superconducting systems, to the thermal fluctuations of alkali metal atoms in fullerene superconductors, and perhaps to the analysis of order-disorder transitions involving deuterated groups based on neutron powder data collected at the HFBR.

Technical Progress and Results: The work described in this report has been carried out by Dr. Robert Papoular, a Visiting Scientist on leave from CEN, Saclay, France. The main thrust of this work has been directed towards three objectives; (i) to locate weak scatterers in inorganic materials where part of the structure is known through the use of entropic Fourier maps rather than traditional difference Fourier maps (ii) to retrieve electron density distributions from high-resolution powder patterns as accurately as possible (iii) to devise optimal experimental strategies for data collection, in particular at a synchrotron beamline such as X7A. In addition, some preliminary work was done on two other applications of MaxEnt, the retrieval of pair-distribution functions for the determination of local order (in collaboration with professor T. Egami, U. of Penn) and accurate deformation densities in chemical bonds from single crystal data (in collaboration with Professor P. Coppens, SUNY at Buffalo).

A user-friendly program (XRPOWDER) was written to analyze high-resolution x-ray powder diffraction data sets such as those obtained on X7A at the NSLS. This program utilizes the commercial MEMSYS library routines developed by Skilling and colleagues

at Cambridge, the input files being either standard data files from X7A or other simple crystallographic files. The basic idea behind the program is to use both the non-overlapping reflections where a reasonable starting model is used to phase the structure factors, and also the overlapped intensities, in which case the phase information is lost. The integrated intensities to be fitted are obtained via a procedure developed by LeBail and colleagues which has now become a standard technique. Our experience has shown that Bragg peaks can be considered non-overlapping if separated by at least one full-width at half-maximum (FWHM). The phases are obtained by Rietveld refinement of an approximate structural model, which in actual practice can be quite crude. It has been found that a crucial factor is a proper estimation of the experimental error bars on the integrated intensities, and a suitable procedure for this has been devised and tested. A poor estimate of these error bars usually leads to incorrect results.

A more general program (GIFT - Generalized Inverse Fourier Transform) was also written, which uses a two-channel entropy approach. It can be used for neutron data, for deformation densities, or more generally when a sizeable fragment of the crystal to be studied is already known. The first channel is then used to reconstruct either the positive part of the scattering or deformation density, or the known fragment part. The second channel is used to reconstruct either the negative part of the density or the remaining unknown part. If they are known, atomic form factors or Debye-Waller factors can also be introduced, giving then a number-density rather than a scattering-density reconstruction.

These programs are currently running on a 486-PC or a DEC-Alpha station. They have been tested by checking results

previously obtained by Sakata and colleagues for simple compounds such as TiO_2 (both x-ray and neutron data), for which essentially identical reconstructions have been obtained with the same hypotheses.

A wide variety of systems has been investigated, and the plausibility of the conclusions checked by simulations in which the calculated structure factors obtained from an idealized model are used. A new feature obtained from a real data set is considered plausible if not present in the corresponding simulation. Very often, the latter show artifacts that stem from the overlap of Bragg peaks and the resulting loss of phase information. An important conclusion from this is that peak overlap should be reduced as much as possible by the use of high-resolution diffraction optics and well-crystallized samples.

Based on the approach described above, electron density reconstructions have been made from high-resolution powder data obtained on two zeolite samples containing small amounts of extra-framework cations (which are responsible from the catalytic properties), LaB_6 , NH_4Cl , CuO , C_{60} and K_3C_{60} . A few examples of the results are illustrated in the following figures.

PAPERS/JOURNALS/PUBLICATIONS:

Based on the results described above, two papers are in preparation:

Model-free search for cations in zeolites from high-resolution x-ray powder data using the Maximum Entropy Method (R.J. Papoular and D. E. Cox).

The two-channel Maximum Entropy Method applied to the charge density of a molecular crystal: α -glycine (R. J. Papoular, Y. Vekhter

and P. Coppens).

The material of the second paper has been presented as a poster to the SAGAMORE XI CONFERENCE (August 1994, France).

LDRD FUNDING:

FY 1993	\$ 24,431
FY 1994	73,342
FY 1995 est.	40,000

only 42 NON OVERLAPPING reflections
Standard Fourier Synthesis

a)

only 42 NON OVERLAPPING reflections
Maximum Entropy reconstruction

b)

all available 180 reflections
Maximum Entropy reconstruction

c)

ZEOLITE NaA - Section Z=0.10

Fig.1(a). Reconstructed section for a NaA zeolite sample of nominal composition $\text{Na}_x(\text{Si}_{1-x}\text{Al}_x)\text{O}_2$ from a traditional Fourier synthesis based on 42 non-overlapping reflections. The Na atom cannot be reliably located.

Fig.1(b). MaxEnt reconstruction based on the same data. The Si, Al and Na atoms are now clearly seen, but the O atoms are unresolved and some weak artifacts are present.

Fig.1(c). MaxEnt reconstruction based on all the observed peaks, overlapped or not. All the atoms can now be seen, and the weak artifacts have disappeared.

Fig.2. MaxEnt reconstructed section for a zeolite-Y sample ($\text{Si}_{1-x}\text{Al}_x\text{O}_2$) containing small amounts of La and Pd. All the contours represent significant scattering density which can be used to modify the model for Rietveld refinement.

B atoms in section $Z=1/2$ - LaB_6

Fig.3. MaxEnt reconstruction of the boron plane in the structure of LaB_6 . Despite boron being a very weak scatterer (5 electrons versus 57 for La) and the severe peak overlap (~70%), this section is reconstructed with very high signal-to-noise discrimination, and shows no evidence of any distortion of the electron density, in agreement with a previous detailed single-crystal study.

NH_4Cl - Room temperature

Fig.4. Location of H atoms in NH_4Cl based on a MaxEnt reconstruction from x-ray powder data collected at 298 K, slightly above the order-disorder transition temperature. The H atoms are well-localized and show no evidence of large dynamic fluctuations.

α -GLYCINE: Standard Fourier

a)

α -GLYCINE: Maximum Entropy

b)

Fig.5 Reconstruction of scattering density for a small organic molecule, α -glycine, from single-crystal data (1206 Bragg peaks) based on (a) standard Fourier synthesis (b) two-channel MaxEnt. Note the enhanced detail obtained for the deformation density. (In collaboration with P. Coppens).

Accelerator Technology

A. G. Ruggiero

93-30

PROJECT DESCRIPTION:

It is important to investigate new methods and applications of the Accelerator Technology in order to resolve limitations of the present state of the art and to explore advanced technologies which may be available in the near future. The purpose of the study is to find out the feasibility of projects of different nature and application and yet possibly interconnected to each other. More specifically our interest is around four major topics: - A large-energy Muon Collider for the search of the Higgs boson; - The properties of Crystalline Beams and the conceptual design of a very low-energy storage ring where to attempt forming, to observe and to experimentally investigate the Crystalline Beam; - Methods of producing and controlling Nuclear Energy by Fusion of Light Ions, like proton and boron, in clean and small-size environments; - Feasibility Study of an intense Spallation Neutron Source obtained by impinging an intense medium-energy proton beam on a target. The first item deals with a topic of High Energy Physics which is of paramount importance, and that will play an important role during the next ten or twenty years. The second is basic research that considers the creation of a very cold state of matter resulting from very sophisticated techniques of beam cooling and diagnostics. The production of nuclear energy in a clean and modest size environment is the goal of the third topic which considers methods of colliding beams different from those used or being proposed for the magnetic or inertial confinement. Finally, the last topic of research explores the methods of accelerating proton

beams to the energy of several GeV with an average intensity two orders of magnitude larger than presently achieved in conventional accelerators. Each of these studies have crucial accelerator physics and technology issues which are important to be investigated analytically and numerically. The muon-collider project requires the demonstration of production, acceleration and cooling over a period of time of a couple of milliseconds to avoid depletion of the beam intensity due to the short particle lifetime. The Crystalline Beam requires study of controlling space-charge and intrabeam scattering effects as well advancing the limits of cooling techniques. The production of nuclear fusion energy by colliding beams requires also the generation of very intense beams and thus the experimental demonstration of controlling space-charge effects by gas-neutralization, of electron and laser cooling at very low energies and of thermalization techniques. The study of intensity limitations and of the methods to cope with space-charge effects is the common denominator, together with the cooling techniques, to all our projects including the feasibility study of the Spallation Neutron Source. In the latter we explore the technical capability of the components, like rf and magnet systems, of fast cycling synchrotrons.

TECHNICAL PROGRESS AND RESULTS - Fiscal Year 1994:

Purpose: We describe herewith the purpose of research for each of the four topics.

- Determination of a conceptual scenario for a muon collider based on the production of μ particles with an intense beam of protons which could be available with an upgrade of the BNL-AGS facility. Also determination of the need, requirements and limitations of a very fast stochastic cooling technique to reduce the beam betatron emittance at the collision point.

- Investigation of the fundamental properties and structure of the Crystalline Beam in the ultimate state and the requirements of the magnet lattice of the storage ring where it is to circulate.
- Conceptual design of a very low energy storage and collider ring where the collision head-on of two intense beams of protons and of ions of boron partially stripped takes place. Calculation of the requirements on the beam intensity and dimensions for an efficient production of nuclear fusion energy around a 10 kWatt level. Studies of Laser Cooling feasibility.
- Preliminary feasibility study of a 5 MWatt Pulsed Spallation Neutron Source based on the use of a 600 MeV Linac and two fast cycling Synchrotrons for the acceleration of protons to 3.6 GeV. Determination of the beam performance and of the limitations due to the large intensity.

Approach: This is described below for each of the four areas of research.

- The conceptual study of the muon collider has been done in collaboration with about a dozen of scientists in USA. The subject was discussed during the Workshop on Beam Cooling and Related Topics in Montreux, on October 1993. Meetings on this topic were also held at Lawrence Berkeley Laboratory during 1994. We investigated a possible scenario and the requirements of stochastic cooling both analytically and numerically.
- We performed analytical and computational study to determine ground states of Crystalline Beams in low-energy ion storage rings. Crystalline Beams have been important topic of discussion at the Montreux Workshop (Oct. 1993), at the Heidelberg Workshop (July 1994) and in few meetings held at the Laboratori Nazionali di Legnaro in Italy, that has an interest in building a storage ring

dedicated to the demonstration of such beams.

- We developed a scenario of extracting energy from the nuclear fusion reaction between a beam of protons and a beam of ions of boron, using a novel concept of circular radio-frequency quadrupole, and fast laser cooling which freezes both beams in their ground crystalline state.

- A BNL study group was appointed with the task of exploring the feasibility of a 5 MWatt Pulsed Spallation Neutron Source. As a member of the study group we had the opportunity, through a sequence of regular meetings, to collaborate for the preparation of a preliminary design of the facility and the study of the beam performance and of some of the components of the accelerators. We had also an opportunity to test for this study a software that we have prepared in the meantime for the design of acceleration cycles in circular machines.

Technical Progress and Results: After having worked out an upgrade scenario of the AGS facility for the production of intense muon beams, we have studied the feasibility of fast stochastic cooling in a single-pass mode with the help of powerful non-linear magnets and of modulation of the amplifier gain, for instance by reducing the pickup plate separation as cooling proceeded to enhance the sensitivity and the signal-to-noise ratio. It was found that in any case a serious limitation emerges due to the finite temperature of the preamplifiers which causes a finite betatron size considerably larger than required. A different cooling system has been investigated which is based on the concept of using optical amplification at the 10^{14} Hz frequency. The radiation, which provides the signal with the information of the beam size, is obtained by the motion of the muons in a quadrupole undulator magnet, amplified and applied back

to the particles in a dipole undulator. Considering the very short wavelength, considerably smaller than the bunch length, the beam dynamics is now of different nature and it seems that indeed large cooling rate can be obtained. Moreover, very little thermal noise is expected from the optical amplifiers, which makes the method feasible for cooling muons. A colliding beam scenario at large energies has been investigated with a luminosity in excess of $10^{30} \text{ cm}^{-2} \text{ s}^{-1}$. The results will be described in the technical note presently under preparation, presented and discussed at the Sausalito meeting on November 1994.

- We developed a fully analytical approach to the study of Crystalline Beams in storage rings, in terms of such fundamental accelerator concepts as tune shift and stopband. We considered a Crystalline Beam consisting of substrings, arranged symmetrically around the reference trajectory, and we examined the motion of a slightly perturbed test particle on one of them. Our approach quite naturally leads to the conclusion, that storage rings need to operate below the transition energy, and that Crystalline Beams have the same periodicity of the storage ring. Each Crystalline Beam has an upper and lower limit of the spacing between the ions. The upper limit is set by the periodicity condition, and the lower limit is set by the stability of the test particle motion around the equilibrium. Crystalline Beams represent an interesting concept which can allow to overcome limitations in storage rings due to space charge forces and to Coulomb intrabeam scattering. An interesting application is the enhancement of the luminosity of colliding beams by properly aligning the substrings on each other. A computer program has been prepared which evaluates and determines the ground states of Crystalline Beams in a given storage ring as function of beam parameters and focussing of the ring. Several technical notes and summary and review reports have been prepared as well

as communications to workshops and to conferences. Recently we have been granted authorization and financial support from prof. A. Zichichi, director of the E. Majorana Cultural Center, to organize a Workshop on Crystalline beams and Related Issues in Erice, Sicily, on November 1995 within the framework of the Eloisatron Project.

- As a consequence of the above study we have immediately perceived the relation between Crystalline Beams and the low-energy collider ring where nuclear fusion can be produced by colliding a beam of protons (0.37 MeV) with a beam of ions of boron (0.31 MeV). The storage ring has a relatively small dimension with a diameter of about 2.5 meter, where the two beams collide continuously head-on along the common circumference. The storage ring is a Circular Radio-Frequency Quadrupole made of four parallel copper rods among which a transverse electromagnetic mode is excited. Continuous current flowing along the outer rods provides a magnetic field that bends both beams on the common circular orbit. Beam Crystallization is proposed as the method of increasing ion-beam densities and, thus, the rate of fusion events exceeding the space-charge and interparticle Coulomb interaction limits. The circular radio-frequency quadrupole represents a novel concept for which we shall seek patenting. We shall want to patent also the whole proposal of producing nuclear fusion power in the relatively small amount of 10 kW, which is just the need of a typical household in the United States.

- As we have seen, a relation between the studies of a Muon Collider and of a Spallation Neutron Source has been evidenced since both requires an intense proton source of about the same energy and intensity. We have found that a facility based on the use of a 600 MeV Linac followed by a pair of fast cycling Synchrotrons is indeed technical feasible. The demonstration of feasibility of this scenario required the

numerical investigation of the rf capture during injection, the simulation of injection of hundreds of beam turns of negative ions, the design of the rf cavity system for the acceleration, the analysis of the ramping of the guiding field at a large rate and the impact to the vacuum system. A feasibility study report has been prepared which is ready for distribution. Results have been compared and discussed with other study groups in US and Europe.

PAPERS/JOURNALS/PUBLICATIONS:

A. G. Ruggiero, Stochastic Cooling Requirements for a Muon Collider, Proceedings of the Workshop on Beam Cooling and Related Topics, Montreux, Switzerland, Oct. 4-8, 1993, p. 152. CERN 94-03.

S. Chattopadhyay, ..., A. G. Ruggiero, ..., Critical Issues in Muon Colliders, Proceedings of the Workshop on Beam Cooling and Related Topics, Montreux, Switzerland, Oct. 4-8, 1993, page 439, CERN 94-03.

A. G. Ruggiero, A muon collider scenario based on stochastic cooling, Nucl. Instr. and Meth. in Phys. Res. A 00 (1994) NIM01322 T.

A. G. Ruggiero, Optical Stochastic Cooling for a Muon Collider, BNL Internal report in preparation, to be presented to the Sausalito Workshop, Nov. 17-19, 1994.

A. F. Haffmans, D. Maletic and A. G. Ruggiero, Crystalline Beams ..., Internal Reports BNL - 60436 / 60619 / 60743 / 60876, April - June, 1994.

A. F. Haffmans, D. Maletic and A. G. Ruggiero, Study of the Effect of space Charge using SYNCH, Internal Report BNL - 60521, April 1994. Also presented to the 1994 European Particle Accelerator Conference, London, UK.

A. F. Haffmans, Confinement and Stability of Crystalline Beams in Storage Rings, to appear in the proceedings of the 6th Workshop on Advanced Accelerator Concepts, Wisconsin, June 12-18, 1994.

A. G. Ruggiero, Crystalline Beams: Theory, Experiments and Proposals, to appear in the proceedings of the INFN Eloisatron Workshop on Quantum-like Models and Coherent Effects, Erice, Sicily, June 13-20, 1994.

A. F. Haffmans, D. Maletic and A. G. Ruggiero, Matrix Formulation of the Particle Motion in Crystalline Beams, presented to the 1994 European Particle Accelerator Conference, London, UK.

A. G. Ruggiero, Concept of a Nuclear Plant based on the Fusion reaction of protons with Ions of Boron, BNL Internal Report in preparation, Sept. 1994.

A. G. Ruggiero, Study of a Spallation Neutron Source based on Fast-Cycling Synchrotrons, presented to the 1994 European Particle Accelerator Conference, London, UK.

5 MW Pulsed Spallation Neutron Source. Preconceptual Design Study. BNL - 60678. June 1994.

CRYSTAL. A Storage Ring for Crystalline Beams. Feasibility Study. LNL - INFN (Rep) 80/94.

FOLLOW-ON FUNDING :

We shall seek support for the muon-collider studies from the DOE-HEP Division. We shall prepare a proposal on the combined studies of Crystalline Beams and Nuclear Fusion to be submitted to the cognizant DOE Division or to other independent concerns, including industries. We also expect to receive support

for the study of the PSNS during the upcoming years from DOE.

LDRD FUNDING :

FY 1993	\$153,210
FY 1994	249,900

Analysis of Structures and Interactions of Nucleic Acids and Proteins by Small-Angle X-Ray Diffraction

M. Capel

93-32

PROJECT DESCRIPTION:

Small-angle x-ray and neutron scattering are being used to examine the structure of large complexes of biologically relevant macromolecules in solution.

TECHNICAL PROGRESS AND RESULTS - Fiscal Year 1994:

Purpose: Many biologically important processes are mediated by large complexes of proteins, or proteins and nucleic acids. For example, protein synthesis and folding are mediated by the ribosome and chaperonins respectively; while ATP-dependent proteases mediate degradation of damaged proteins. In most cases, these complexes have not yielded to structure determination by x-ray crystallography, thus limiting our understanding of their function. We plan to develop techniques for studying the low resolution structures of large complexes in solution. Studies of this type will be particularly useful for systems where high resolution structures for the isolated components can be obtained. In these systems, the combined use of low-resolution studies for the complexes and high-resolution studies of the components will provide a framework for understanding the function of the complex. A major limitation is in extracting the contribution of a individual subunit or component. As a means of overcoming this difficulty the contribution of

individual components will be enhanced by selectively labeling the target protein or nucleic acid with either gold clusters, for x-ray scattering studies, or uniformly labeling with deuterium for neutron scattering. The scattering curves for complexes reconstituted with labeled and unlabeled components can then be compared to gain information about the location of the labeled component.

Approach: Three systems have been chosen to test these techniques. These are the *Escherichia coli* Hsp60 and Hsp70 chaperonin and the ATP-dependent protease Clp systems. To ensure that the structural studies are biologically relevant, both biochemical and genetic studies will also be conducted in parallel. The proposed structural studies require a large amount of purified protein, and to this end each of the components have been cloned and over-expressed. In some cases, cysteine residues have been introduced into individual proteins to facilitate their labeling with gold clusters. For proteins having a free cysteine, labeling can be achieved by reacting the proteins with sulfhydryl-specific gold clusters.

Technical Progress and Results: (a) We have examined the structure of the Hsp60 chaperonin system by neutron and x-ray scattering. This system is formed by two proteins, GroEL and GroES that together are important for the folding of proteins both *in vitro* and *in vivo*. GroEL is a tetradecamer of 57 kDa subunits and GroES is a heptamer of 10 kDa subunits. A tight complex is formed between GroEL (801 kDa) and GroES (70 kDa) in the presence of the nucleotides ATP or ADP, but not in their absence. To determine the stoichiometry and location of GroES in the complex GroES was uniformly labeled with deuterium, thereby increasing its contribution to the neutron

scattering of the complex in $^1\text{H}_2\text{O}$ solution. The stoichiometry of the ADP-GroEL/GroES complex was found to be 1:1; with GroES being located at the periphery. Moreover, by matching the contribution of deuterated GroES to that of the solvent, we have demonstrated that GroES does not induce a large conformational change in GroEL. This latter observation has important implications for the function of the GroEL/GroES chaperonin system.

(b) Structural studies have also been initiated with the *E. coli* Clp ATP-dependent protease system. The Clp system is composed of two subunits; ClpP, the protease subunits, and ClpA which is required for recognition and presentation of unfolded substrates to ClpP. This system appears to be functionally and structurally related to the 24S proteasome found in most eucaryotic cells. Initial small-angle x-ray and neutron scattering studies indicate that ClpP exists as a dodecamer in solution. The structure of the ClpP oligomer is best described as a hollow cylinder having a central axial pore of 36 Å. The central pore is sufficiently large to accommodate a portion of an unfolded protein. Studies of the ClpA component have only recently been initiated. The monomeric molecular weight of ClpA is 97 kDa; however, it is thought to act as an oligomer of unknown stoichiometry. ClpA possesses an intrinsic ATPase activity that can be stimulated by unfolded substrates. In the presence of ATP, ClpA and ClpP associate to form the active complex. Studies are currently in progress to determine the molecular weight and low resolution structure for the functional ClpA complex, and also for the ClpA/ClpP complex. During the course of this work a novel fluorescently labeled protease substrate (rhodamine-casein) has been identified. This substrate may replace the radioactively labeled substrate currently used to assay for Clp function.

(c) Studies are also in progress to examine the structure/function of the Hsp70 chaperonin system, which in *E. coli* is made up of the proteins DnaK (70 kDa), DnaJ (40 kDa), and GrpE (20 kDa). The Hsp70 and Hsp60 chaperonin systems are thought to act sequentially in the protein folding pathway *in vivo*. The mechanism of action for the Hsp70 system is not as well characterized as for the Hsp60 system, and thus studies have been undertaken to characterize this system. Initially it has been observed that DnaK can interact with short hydrophobic peptides, and the unfolded state of a few proteins. Our recent studies indicate that the biologically relevant functional complex involves both DnaK and DnaJ (DnaK-substrate-DnaJ). Further, the formation of the ternary complex requires a single round of ATP hydrolysis by DnaK. These studies have led to the isolation of a complex containing GrpE-ADP-DnaK-substrate-DnaJ that is stable and biologically active in protein folding. This complex can also be produced in sufficient quantities for structural studies. In parallel, high resolution structural studies of DnaJ have been initiated. These studies have led to a NMR based model for the N-terminal DnaJ homology domain. Ultimately, we hope that the combined use of low-resolution structures of the complex and high-resolution structural studies of the individual components will aid in understanding the mechanism for this important chaperonin system.

PAPERS/JOURNALS/PUBLICATIONS:

R. W. Kriwacki, R. B. Hill, J. M. Flanagan, J. P. Caradonna, and J. P. Prestegard. New NMR methods for the characterization of bound waters in macromolecules. *J. Amer. Chem. Soc.* 115, 8907-8911 (1993).

A. Szabo, T. Langer, H. Schroder, J. Flanagan, B. Bukau, and F.-U. Hartl. The ATP hydrolysis-dependent reaction cycle of the *E. coli* Hsp70 system, DnaK, DnaJ, GrpE. *Proc. Natl. Acad. Sci. USA* (in press).

R. B. Hill, J. M. Flanagan, and J. Prestegard. ¹H and ¹⁵N magnetic resonance backbone assignments and secondary structure of *E. coli* DnaJ(1-78). Submitted to *Biochemistry*.

J. M. Flanagan, M. Capel, and J. Shanklin. The *E. coli* protease ClpP exists as a dodecamer in solution. Manuscript in preparation.

V. Graziano, M. Capel, D. Schneider, and J. M. Flanagan. Solution scattering on complexes of GroES/GroEL: Absence of a large-scale conformational change in GroEL upon binding of GroES. Manuscript in

preparation.

A. Szabo, R. Korszun, F.-U. Hartl, and J. M. Flanagan. *E. coli* DnaJ contains a zinc-finger motif which is important for substrate binding. Manuscript in preparation.

FOLLOW-ON FUNDING:

A National Institutes of Health proposal entitled, "Structural and Functional Studies of the *E. coli* Chaperone DnaJ" was submitted on October 1, 1994.

LDRD FUNDING:

FY 1993	\$42,276
FY 1994	97,690
FY 1995 est.	48,000

Enhancement of Microplanar Beam Radiation Therapy (MRT) of the Intracerebra Rat 9L Gliosarcoma by Preinfusion with a Diiodofluorescein-Histone Conjugate

D.N. Slatkin, M. Miura
and P. Spanne*

93-33

PROJECT DESCRIPTION:

The original statement of work (in LDRD Annual Report, December 1993, BNL-52351, pp. 47-49) envisioned the synthesis of histone H3 (cysteine 110)-acetylamino(4',5' diiodo) fluorescein [H3AADIF] and the study of enhancement of MRT by prior perfusion with H3AADIF. Later, it was decided to include ^{157}Gd as a possible radiation therapy enhancement agent *via* an amide linkage of a Gd-DTPA chelate to a nucleopetal aminated derivative of H3AADIF.

TECHNICAL PROGRESS AND RESULTS -Fiscal Year 1994

Several technical trials determined that a miniature (1-2 ml) culture of 9L gliosarcoma cells could be initiated in a small shallow flat-surfaced plastic dish in such a way that it became confluent $\approx$ 48-96 hours after inoculation and that it could be examined by high power fluorescence microscopy using a standard, upright microscope by sealing the lid of the dish with Vaseline and inverting the dish to examine cells adhering to its bottom. This examination did not materially affect the viability of the culture. Thus it would be

feasible to follow, in individual cells, the cytoplasmic and nuclear influx and egress of a fluorescent additive to the growth medium such as H3AADIF would be. However, it became clear that the synthesis and testing of H3AADIF demands a major commitment of time and effort, perhaps a larger commitment of the participants' total effort than would be feasible. Therefore, the research directorate in DOE/OHER was asked to comment on a summary of this project and to judge the likelihood of long-term DOE support for a gadolinium neutron-capture therapy [GdNCT] research program based on the premise of a favorable outcome to the effort. DOE's response to our initiative was unequivocally negative. It was thereupon decided to reconsider in detail the postulate upon which our proposal had been made. We evaluated quantitatively the concept that molecular dimensions of contiguity are required between ^{157}Gd and chromatin to optimally use the electron radiation in GdNCT.

To do this, we took advantage of a report on GdNCT brought to our attention by Robert M. Brugger (G.A. Miller et al., *Nuclear Technology* **103**, 320-331, 1993) in which the probability of occurrence of conversion and Auger electrons over the energy range 0-7 MeV is graphed (Fig. 2, Miller et al.) and the ranges of electrons of these energies in water are tabulated (Table 2, Miller et al.). This graph was analyzed by us with particular attention to determining the importance of electrons with ranges greater than tumor cell dimensions. First, by normalizing the relative frequency of 150 keV electrons to 0.01, it was estimated that the relative frequency of electrons, $P(E)$,

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with energies under 300 keV could be expressed by

$$P(E) \approx \exp(-30.6 E); E < 0.3,$$

where E is the conversion or Auger electron energy in MeV.

With the same normalization,

$$P(E) \approx \exp[-9.21]; 0.3 < E < 1.17.$$

For $E > 1.17$, $P(E)$ dropped again exponentially with a half-frequency energy increment of $\approx .17$ MeV, reaching $\approx 10^{-7}$ at energies more than 2.8 MeV. Miller et al. defined a frequency of 10^{-8} as equivalent to zero and calculated that the average ^{157}Gd conversion/Auger electron energy was 91 keV. Our analysis of Miller et al.'s Fig. 2 then suggested that a ^{157}Gd neutron-capture conversion or Auger electron is several hundred times more likely to have less than 300 keV energy than more. Since 300 keV electrons have a range of over 0.5 mm in soft tissue, we chose to confine our attention to those with energy less than 300 keV, i.e., to the vast majority of electrons produced by ^{157}Gd neutron-capture. The relative amount of energy carried by the subset of electrons in the range E_1 - E_2 can be approximated by

$$\int_{E_1}^{E_2} E \cdot P(E) dE,$$

and the range of 25 keV electrons in water is approximately one tumor cell diameter, 12 μm .

We calculated the ratio of $\int_{.025}^{.3} E \cdot P(E) dE$ to

$\int_0^{.3} E \cdot P(E) dE$ and derived the fraction of all energy carried by < 300 keV ^{157}Gd conversion and Auger electrons that is carried by those with ranges over 12 μm in soft tissue. Our

calculation showed this fraction to be unexpectedly high, 82%. The indefinite integral used for the calculation was:

$$\int E \cdot \exp(-kE) dE = -(kE+1) \cdot \exp(-kE) / k^2$$

where $k = 30.6$.

This result indicated that most of the energy from the conversion/Auger cascade from ^{157}Gd is much more diffuse at the cellular level than we had previously understood, which called into question the likelihood that our initial postulate would be upheld. The dramatic reprise of clinical BNCT on September 13, 1994 at Brookhaven National Laboratory, our unease about the science and logistics of Auger-mediated GdNCT research, together with discouragement from DOE/OHER, led us to put aside the I/Gd-histone approach to our project and thereby to more productively concentrate this LDRD's resources on experimental MRT for the 9L gliosarcoma. Nevertheless, it now appears reasonable to postulate that GdNCT may be nearly as well mediated by a Gd-bearing agent in the cell cytoplasm as in the nucleus. There is perhaps some minor disagreement between published opinions of the relative importance of gamma and electron radiation for GdNCT. Although J.T. Masianowski et al. (in *Advances in Neutron Capture Therapy*, ed. A.H. Soloway et al., Plenum Press, New York 1993, pp. 231-234) recently suggested that electrons would impart only about half as much energy to a tumor as would the gamma radiation, T. Matsumoto (in *Ibid.*, pp. 235-239) indicated that the electron and gamma energy contributions would be similar. Whatever computations will eventually prove most relevant to clinical GdNCT, it is clear that any *in vitro* experiments to initially evaluate the feasibility of GdNCT should require that the

distribution of cell-to-cell distances be known and, therefore, that a cell culture system be in suspension and appropriately agitated during neutron irradiation. One approach could be to develop a Gd-linked porphyrin that, like other porphyrins, concentrates in the cell cytoplasm but might be less toxic than the planar boron-linked porphyrins. If some of the toxicity of porphyrins is associated with a planar configuration that enables it to intercalate between protein and nucleic acid moieties and, at high intracellular concentrations, to disturb metabolic functions, it would be worthwhile to test a Gd-linked member of the non-planar porphyrin family, new members of which have been synthesized and studied by Miura et al. recently¹.

On the average, gamma emission from one neutron-capture by ^{157}Gd has been estimated to impart only 4% as much energy to a uniformly gadolinium tumor as would one neutron-capture by ^{10}B to a uniformly boronated tumor (R.M. Brugger et al., in *Advances in Neutron Capture Therapy*, ed. A.H. Soloway et al., Plenum Press, New York 1993, pp. 225-229). Whereas the 240,000 barn thermal neutron-capture cross section of ^{157}Gd is 62.6-fold greater than that of ^{10}B , the electron energy from neutron-capture by ^{157}Gd , 66 keV (Miller et al., 1993), is on the average 35.5-fold less than the combined alpha and lithium-7 particle energy from ^{10}B . These factors indicate a net 76% energy advantage of ^{157}Gd over ^{10}B . However, the ≈ 3 -fold advantage in relative biological effectiveness [RBE]² of the particles from ^{10}B over the gammas and electrons from ^{157}Gd gives BNCT an intrinsic ≈ 2 -fold radiotherapeutic advantage over GdNCT per targeted atom. The RBE of Auger electrons is greatly enhanced when the decay occurs inside or very near the DNA double helix³. In the special case of neutron-induced ^{157}Gd decay, however, such a significant fraction of the energy of the resultant gamma and electron

radiation is emitted with a range greater than one cell diameter that reproductive cell death might be more readily induced using GdNCT by transporting large amounts of ^{157}Gd mainly to the cytoplasm than relatively small amounts mainly to be in molecular or atomic contiguity with nuclear DNA. From these considerations we surmise that, for combined GdNCT and BNCT, adding one ^{157}Gd atom to each ^{10}B atom delivered to the cytoplasm of tumor cells would be therapeutically nearly equivalent to, and perhaps less difficult to achieve than increasing the intracytoplasmic concentration of ^{10}B in tumor cells by $\approx 50\%$ for BNCT alone. This combination could be more effective for neutron capture therapy [NCT] of extracranial than of intracranial tumors, because an added burden of absorbed dose from ^{157}Gd neutron-capture to the vasculature of normal tissues between and near tongues of extracranial tumor tissue would not be expected to present such severe clinical risks. Thus, it appears appropriate to pursue GdNCT research in parallel with BNCT research, as indicated previously by Brugger et al (1993), using Gd carriers with low toxicity that have high affinity to tumor cell cytoplasm but not necessarily, as we had originally thought advantageous, a high affinity to tumor cell chromatin.

In FY 1994, several publications of work relating or potentially relating to MRT and NCT, of which at least one of us was co-author, have appeared and are listed below. The invention application for MRT (S.N. 052,927; Slatkin, Dilmanian, and Spanne), described in item 1 of our December 1993 LDRD report (BNL-52351; page 49), was granted by the U.S. Patent office⁴ after some initial challenges were presented by the U.S. Patent Office examiner and, in collaboration with legal counsel to the BNL Patent Office, answered to his satisfaction. Ownership of

this Patent (No. 5,339,347; August 16, 1994) has been assigned to the U.S. Department of Energy. In item 2 of the 1993 LDRD report, mention was made of a manuscript entitled "Subacute Neuropathological Effects of Microplanar Beams of X Rays from a Synchrotron Wiggler" (Slatkin et al.), which had been submitted to Dr. E.P. Cronkite for possible communication to the Proceedings of the National Academy of Sciences [PNAS]. That manuscript passed the peer review process during FY 1994. The few deficiencies identified by one of the reviewers selected by Dr. Cronkite were corrected to that reviewer's and to Dr. Cronkite's satisfaction (E.P. Cronkite; personal communication to Slatkin; October 5, 1994). It is expected that Dr. Cronkite will communicate the revised manuscript to PNAS early in 1995. Absent unanticipated hurdles, the revised manuscript is likely to be published in PNAS during 1995.

In addition to the approach to the MRT of the rat intracerebral 9L gliosarcoma⁵ published in BNL-52415 and to be published in 1995⁶, we now present the preliminary results of a slightly different MRT plan for that gliosarcoma. The Kaplan-Meier plot (Fig. 1) shows the percentage of rats in three initially matched, differently treated groups (experiment 94MRT2). In the tridirectionally irradiated groups, each rat received a three-tiered (4 mm height per tier), 1 cm-wide array of parallel microbeams, each array equivalent to 100 25 μ m-wide, 12 mm-high, horizontally propagated microbeams, separated 100 μ m on center. These directions were, anatomically: 1) right lateral, 0°; 2) right anterior oblique, +60°; and 3) left anterior oblique, +120°, the prone rat having been swiveled in the horizontal plane so that each of the three anatomical directions was along the axis of the wiggler-generated beam. As of day 70 after MRT (Fig. 1) none of the 312 Gy-entrance rats had died of recurrent tumor, but rather of progressive cachexia without obvious

signs of motor deficits. At least three of the rats in the 221 Gy-entrance group died with recurrent tumor in the right cerebral hemisphere. Detailed neuropathology for this experiment will be performed by our research collaborators in Switzerland. It should be mentioned that, unlike arrays 1) and 2), the left anterior oblique arrays 3) were not aligned to symmetrically traverse and straddle the presumed isocenter of the gliosarcoma ($\approx$ 4-5 mm deep to the calvarial site of inoculation, $\approx$ 9.60 mm posterior to the mid-interocular line, and $\approx$ 4.00 mm to the right of the midsagittal plane). The midplanes of the left anterior oblique irradiations were inadvertently offset 1.9 mm so that only 80% of the target volume was congruent with the intended target volume. Since a 14 day-old 9L gliosarcoma is $\approx$ 4 mm in diameter⁷, the margin of a left anterior oblique array grazed the left edge of the tumor rather than pass $\approx$ 2 mm medial to the tumor as intended. Another technical aberration in this experiment was that all the rats were offered only drinking water with 0.5% (w/v) piperazine citrate in tap water rather than plain tap water during days 6-13 and 20-27 after irradiation on July 26, 1994 as prophylaxis for pinworm infestation, which was noted as an apparently inconsequential condition in other rats during a routine periodic survey of the Brookhaven Medical Department Animal Care Facility. Since the unirradiated rats died with a median postinoculation survival of 21 days, as expected, interpretation of comparisons between the survivals of various experimental groups would not be seriously distorted by the piperazine treatment. However, it is possible that the Kaplan-Meier lines for the 221 Gy-entrance and 312 Gy-entrance irradiations would have been slightly shifted to the right had arrays 3) been as well aligned as were arrays 1) and 2).

In summary, as of postirradiation day 70, survival rates following the tridirectional 221 Gy-entrance irradiations (Fig. 1) seem to be similar to those following bidirectional 312 Gy-entrance irradiations^{5,6}. This suggests that the principle of seamless, macroscopic (> 1 mm) beam conformational radiosurgery by which the number of directions from which an isocentric target is irradiated is increased in order to increase the steepness of the absorbed dose gradient in the penumbra of the targeted zone will also be applicable to MRT. Optimal MRT of the intracerebral rat 9L gliosarcoma, however, will require refinements in Monte Carlo [MC] treatment planning, in computer-controlled three-dimensional rat positioning, and in noninvasive rat brain imaging that are not yet available to us.

We postulate that, among other determinants of the outcome of rat gliosarcoma MRT, the maximum absorbed doses safely achievable between irradiated microplanes in various crossfired and non-crossfired zones of the rat brain are important. Because the rat skull and vertebral column are small and intricately boned, fine-grained MC dosimetry for x-ray microbeams in the rat central nervous system will be difficult to achieve, but important for MRT research.

Some of the progress made by Spanne during FY 94 toward implementing accurate and, eventually, user-friendly MC computations of dose profiles for various rat brain tumor MRT irradiations is summarized as follows. Spanne became familiar with the results of a National Institute of Science and Technology study at the National Synchrotron Light Source [NSLS] X17 beam line during which discrepancies were noted between measured electron energy spectra and spectra predicted by ITS code-based computations. He then reevaluated the MC computations for a tumor in a human head model of the kind reported by us

previously (Slatkin et al, "Microbeam radiation therapy", *Med. Phys.*, 19, 1395-1400, 1992) and found that some, but not all of the calculated peak-to-valley ratios are dependent on whether the EGS or the ITS code is applied. He sought causes for this discrepancy and, although he did not pinpoint them, surmised that differences in the mathematical physics modeling of electron transport (or, conceivably, in the logic used to transfer the transport models into the two codes) are probably involved.

There has already been a promising spin-off from MRT studies that was supported, in part, by this LDRD initiative. A multislit, variable width collimator was designed specifically for MRT⁶. To a large extent based on that design, a device is being made in Canada under contract with the University of Bern, Switzerland. A Swiss/BNL initiative for MRT research in Europe resulted in one of us (Spanne) accepting a position at the European Synchrotron Radiation Facility [ESRF]** with biomedical synchrotron x-ray microbeam research included among his responsibilities. We anticipate that the Canadian-Swiss collimator will be tested at the ESRF by Spanne in 1995. If the results are favorable, MRT research at Brookhaven will be reoriented toward the use of simultaneous parallel multiplanar irradiations using a multislit collimator of similar design, greatly reducing the time required to irradiate many hundreds of precisely aligned microplanar zones in each rat undergoing experimental MRT.

OTHER COLLABORATORS:

We thank F.A. Dilmanian, X-Y. Wu, J.-O. Gebbers, and J.A. Laissue for their permission to describe some preliminary aspects of our joint experiment 94MRT2 in this report. We are indebted to M.M.

Nawrocky for meticulous cultures of 9L gliosarcoma cells. We thank F.A. Dilmanian for reviewing our calculus and A.L. Ruggiero for timely secretarial assistance. We thank J.A. Coderre for constructing Fig. 1.

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LDRD FUNDING:

FY 1993	\$ 42,638
FY 1994	96,966

Relaxographic MRI and Functional MRI

Joanna S. Fowler and 93-35
Charles S. Springer, Jr.

PROJECT DESCRIPTION:

This project began in October, 1993. It consists of three separate components:

The design and development of a high-field Magnetic Resonance Imaging (MRI) laboratory for the study of humans and animals at Brookhaven National Laboratory (BNL).

The continued development of the new form of MRI - *relaxographic* MRI - originated by Professor Springer's research group at the State University of New York at Stony Brook (USB).

The extension of a collaboration between Professor Springer's research group and Dr. Nora D. Volkow of the BNL Medical Department in the *functional* MRI (fMRI) studies of anesthetized mice in Professor Springer's animal MRI laboratory in the USB Department of Chemistry.

TECHNICAL PROGRESS AND RESULTS - Fiscal Year 1994

Purpose: The positron emission tomography (PET) laboratory in the BNL Chemistry Department is world-renowned. It is one of the earliest of all PET labs, and many important (and now frequently used) PET techniques, particularly for the brain, were introduced

at BNL. In the last ten years, the rapid development of MRI brain studies, of physiology and function as well as anatomy, has made it an important complement to PET. Together, these two modalities now dominate the practice of *in vivo* neuroscience. It is essential for the vitality of the new *Brookhaven Center for Imaging and Neuroscience* that it have active laboratories for both techniques; so that they can be used synergistically and, indeed, that their respective data can be intrinsically combined.

Approach: Our approach to accomplish this goal has been greatly facilitated by support from the LDRD Program. Professor Springer began a sabbatical leave from USB in the Fall of 1993. He spent a part of each week at BNL. His salary for the Fall Semester was paid by USB. In January, 1994, he moved to the BNL Chemistry Department full time. His salary for the Spring Semester and for the summer was paid from this LDRD project budget. He spent part of each week at USB in order to maintain his small-animal MRI laboratory there and to supervise his research group of USB Chemistry graduate students.

Technical Progress and Results: We have made significant progress in each of the three components of this project.

Component 1. MRI Laboratory

We have successfully completed negotiations with Siemens Medical Systems, Inc. and its partner, Spectroscopy Imaging Systems (a subsidiary of Varian Associates, Inc.), for the purchase of a very powerful general purpose four Tesla (4 T) human MR

instrument. This is a tremendous accomplishment for BNL. The field strength (measured as flux density) of 4 T for the MR magnet is the largest used for humans. At this time, there are only three other instruments in the world with human-sized magnets of this strength. The \$3.54 million cost of this instrument has been generously supported by OHER in DOE and the National Institute of General Medical Sciences of NIH. At the same time, we have completed the design and let a contract (with M. H. Kane Construction, Inc.) for the construction of a 3,500 square foot building to house the instrument and laboratory. The \$980,000 cost of this facility has been generously supported by the GPP fund of the BNL Director's office. We expect the laboratory and instrument to be operating sometime during the second year of this LDRD project (FY1995).

Component 2. Relaxographic Imaging

We have revised and had accepted a paper introducing and describing the concept of a new fundamental form of MRI, *relaxographic* MRI. This is the production of an image of the spatial distribution of the nuclear spins that comprise only a portion of the distribution of relaxation times that describes the recovery of the entire spin magnetization of an object after an RF pulse. The sum of all relaxographic images of an object is the spin-density image of the object: that is, relaxographic images are spin-density complements. This approach has great power for *in vivo* MR. By far the strongest MR signal from biological tissue is that of the water protons, $^1\text{H}_2\text{O}$. We have shown that relaxographic imaging allows discrimination of this signal into its two basic components; that from

extracellular water and that from intracellular water. Among other things, this may allow the discrimination between intracellular and extracellular edema: the swelling due to influx or efflux of cell water, respectively. What's more, our approach allows the quantitative measurement of the dynamic equilibrium kinetics of the exchange of the water spins between these two fundamental compartments. We have published three refereed abstracts describing our recent work on this subject. Although our initial approach employed a commonly used MRI contrast reagent (CR) to accomplish the discrimination of the intracellular and extracellular water proton signals, we have preliminary data from cell suspensions suggesting that we may be able to do this with only the use of noninvasive pulsed magnetic field gradients. This would be an extremely important development. For example, we could combine such MRI data with PET data in an intrinsic fashion to produce maps of the thermodynamic concentrations of intracellular metabolites or extracellular drug species in the human brain. This has never before been accomplished and is so fundamental that it will almost certainly be of great usefulness. The inclusion of PET data allows the detection of minuscule concentrations of an extremely large variety of substances.

Component 3. Functional Imaging

The term *functional* MRI (fMRI) denotes the direct, noninvasive detection of brain activity by MRI. We have extended our studies on the photic stimulation of anesthetized mice. We have published one refereed abstract and are preparing a manuscript for submission to a journal. We have directly observed

for the first time the effects of anesthesia on the brain's processing of visual information. Also, we believe that we have made the first direct detection of the operation of the "M" visual channel in the vertebrate. This is the channel responsible for the discerning of, among other things, motion in the visual field. It is quite possible that it is the effect of the anesthetic that has allowed us to discriminate this channel from the operation of the "P" channel, which usually obscures the activity of the M channel. The stipend of the graduate student Research Assistant who has been conducting these experiments, Mr. Wei Huang, has been supported by this LDRD project.

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"CR-Free Discrimination of Intra- and Extracellular H_2O Signals from Yeast Cell Suspensions by Diffusion-Space Relaxography (Diffusigraphy)", G. Vetek, I. Palyka, C. H. Sotak, and C. S. Springer, *Abstr., 2nd Mtg. Soc. Magn. Reson.*, Soc. Magn. Reson., Berkeley, CA; p. 1051 (1994).

"Monitoring of Hyperosmotic Blood-Brain-Barrier Opening to Contrast Agent in Rats by Relaxographic Imaging", B. J. Dardzinski, I. Palyka, W. Reith, C. S. Springer, and C. H. Sotak, *Abstr., 2nd Mtg. Soc. Magn. Reson.*, Soc. Magn. Reson., Berkeley, CA; p. 1382 (1994).

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Manuscript in Preparation:

"Functional MRI of the Murine Brain Response to Light: Effects of Anesthesia Level and Stimulus Intensity Changes", W. Huang, H. Li, I. Palyka, E. M. Eisenstein, N. D. Volkow, and C. S. Springer, in preparation.

Presentations:

"Principles of Bulk Magnetic Susceptibility and Relaxographic Imaging," NMR Departmental Seminar; Fox Chase Cancer Center; Philadelphia, PA; 5 October, 1993.

"Functional and Relaxographic Magnetic Resonance Imaging," Brain Mapping Meeting; Department of Energy Facility; Germantown, MD; 7 October, 1993.

"Functional MRI and Relaxographic MRI," Departmental Seminar; Department of Physics and Astronomy; University of Massachusetts; Amherst, MA; 20 October, 1993.

"Relaxographic Analysis of ^{23}Na Signals," 3rd International Workshop on "Molecular Environment of Intra and Extracellular Sodium"; Mikawa Heights, Aichi, Japan; 26 October, 1993. (Oral presentation by Dr. J-H. Lee).

"Molecular Models for Interpreting ^{23}Na Relaxation," 3rd International Workshop on "Molecular Environment of Intra and Extracellular Sodium"; Mikawa Heights, Aichi, Japan; 26 October, 1993. (Oral presentation by Dr. W.D. Rooney).

"A One-Spin, Four-Level Quantum Mechanical System in Living Tissue: ^{23}Na NMR," Chemistry Department Seminar; Brookhaven National Laboratory; Upton, NY; 17 November, 1993.

"Relaxographic MR Imaging," Medical Department Seminar; Brookhaven National Laboratory; Upton, NY; 2 February, 1994.

"Relaxographic MR Imaging," Biomedical Engineering Department Seminar; Worcester Polytechnic Institute; Worcester, MA; 28 March, 1994.

"Signal Intensity Quantitation (Contrast Agents)," Second Meeting of the Society of Magnetic Resonance; San Francisco, CA; 8 August, 1994. (Invited plenary address).

"Monitoring of Hyperosmotic Blood-Brain-Barrier Opening to Contrast Agent in Rats by Relaxographic Imaging," Second Meeting of the Society of Magnetic Resonance; San Francisco, CA; 8 August, 1994. (Poster presentation by Ms. I. Palyka and Mr. B. Dardzinski).

"The Implementation of Longitudinal Relaxographic Imaging with an EPI-Based Pulse Sequence and at Clinical Field Strength," Second Meeting of the Society of Magnetic Resonance; San Francisco, CA; 8 August, 1994. (Poster presentation by Ms. I. Palyka and Mr. B. Dardzinski).

"Functional MR Imaging of the Brain Response to Light Intensity Changes," Second Meeting of the Society of Magnetic Resonance; San Francisco, CA; 10 August, 1994. (Poster presentation by Mr. W. Huang).

"CR-Free Discrimination of Intra- and Extracellular $^1\text{H}_2\text{O}$ Signals from Yeast Cell Suspensions by Diffusion-Space Relaxography (Diffusigraphy)," Second Meeting of the Society of Magnetic Resonance; San Francisco, CA; 10 August, 1994. (Poster presentation by Mr. G. Vetek).

LDRD FUNDING:

FY 1993	\$ 8,675
FY 1994	13,408
FY 1995 est.	85,000

LDRD FUNDING:

FY 1993	\$ 8,675
FY 1994	13,408
FY 1995 est.	85,000

Note: This project involves animal vertebrates.

Very Low Temperature Infrared Laser Absorption as a Potential Analytical Tool

Gregory E. Hall and
Trevor J. Sears

94-03

PROJECT DESCRIPTION:

Infrared laser absorption spectroscopy of molecules at temperatures close to 4 K is being studied in a novel collisionally cooled absorption cell. Calculations indicate that there are large benefits to be gained in terms of sensitivity and measurement accuracy compared to experiments at ambient temperatures. Measurements are being conducted in prototype systems in order to verify these predictions and characterize the samples. The technique has strong potential for development as an analytical tool as well as providing information of fundamental scientific interest.

TECHNICAL PROGRESS AND RESULTS - Fiscal Year 1994:

Purpose: Spectral congestion caused by an abundance of overlapping lines frequently limits the value of spectroscopic measurements. At room temperature, the infrared absorption spectra of gaseous molecules consist of many Doppler-broadened vibration-rotation transitions. Cooling the sample to cryogenic temperatures can have a dramatic effect. The number of lines will decrease, as the number of thermally populated rotational states decreases. The intensity of the remaining lines, belonging to the lowest energy states,

will increase correspondingly. Each line will also become sharper, as the molecules slow down and have a decreased Doppler width. Cooling from 300 K down to 4 K will decrease the line widths by an order of magnitude. For a typical molecular species of interest, C_2H_5 , the ethyl radical, containing two heavy atoms, the rotational partition function decreases from approximately 20,000 at 300 K to less than 20 at 4 K leading to a further factor of 10^3 in absorption intensity for low lying levels. The peak absorption intensities can thus be enhanced by a factor of 10^4 , allowing greater sensitivity, resolution and measurement accuracy.

Approach: Simply cooling a sample to 4 K, however, does not achieve the enhancement described above, since the equilibrium vapor pressure of virtually all molecular species at 4 K is close to zero. In the condensed phase, all the detailed spectral information present in the gas phase spectrum is lost. Recently however, a novel technique has been demonstrated that allows the measurement of gas phase molecular spectra at cryogenic temperatures. In this technique, the molecule of interest is introduced into a low pressure sample of helium gas, held at approximately 4 K, via an insulated needle injector that connects the low temperature cell containing the helium to an external ambient temperature reservoir. The injected molecules suffer collisions with the cold helium atoms and reach the temperature of the cold buffer gas in fewer than 100. Naturally, the introduced molecules eventually diffuse to the walls of the chamber and condense, however this process involves many more collisions, typically 10,000 at 10 mTorr pressure, and a steady state concentration of cold molecular species can be maintained.

Technical Progress and Results: We have constructed an absorption cell from high conductivity copper which is held in the center of a high vacuum chamber. The cell is cooled by an open cycle liquid helium refrigerator and, once cooled, can be maintained at temperatures close to 4.2 K using 1-2 liters of liquid He per hour. The infrared laser light is introduced into the cell via two CaF_2 windows and, in the current design, makes just one pass through the cooled sample region. We have made measurements on nitric oxide (NO) and nitrous oxide (N_2O) in proof-of-principle experiments. These are light molecules, where the gain on cooling is expected to be less than is possible in polyatomic species, but they have the advantage that they are simple to introduce into the cooled cell and have spectra which are easily interpretable, even at room temperature, and so can be used to characterize the degree of cooling in the sample.

Figure 1 R(1) line in the $\text{N}_2\text{O } \nu_3$ fundamental near 2220 cm^{-1} at 300 K (top) and 10 K. The weaker lines in the former are due to a bending hot band.

Examples of the quality of the data are shown in figs. 1 and 2. The first shows the R(1) line region in the ν_3 fundamental of

N_2O taken at ambient (top trace) and at low temperatures. The encouraging results are that the sensitivity of absorption in the cold sample is at least as good as for a similar one at ambient temperature and the expected decrease in Doppler broadened line width is observed. In fact, the observed line widths are not quite as narrow as would be expected based on the sample translational temperature alone because of a contribution from the laser line width which is of the order of 5-10 MHz. The two weaker hot band lines in the ambient temperature spectrum disappear in the low temperature one because they derive from levels with approximately 550 cm^{-1} of internal energy in vibration, which is effectively cooled out under these conditions.

Figure 2 Low-J, Q-branch region of the $\text{NO } ^2\Pi_{1/2}$ vibrational fundamental near to 1876 cm^{-1} observed in the low temperature cell.

Figures 2 and 3 illustrate some results obtained for NO. Here the rotation-vibration lines are split by various fine and hyperfine couplings associated with the unpaired electron spin in the molecule. These splittings are usually masked in the infrared spectrum due to the Doppler broadened line widths, however, they are quite clearly visible in the low temperature spectrum as shown in these figures.

Figure 3 R(0.5) line in the NO vibrational fundamental. Splittings are caused by a combination of fine and hyperfine structure.

From a study of the line intensity distribution in the low temperature spectra, we could estimate a rotational temperature of approximately 15 K in both N₂O and NO in the current cell. The results are very

encouraging and we plan to extend the work to heavier species where the benefits of reduced sample temperature will be much greater and also attempt to study chemically reactive species following ultra-violet-laser-initiated photochemistry in the low temperature environment. Currently, we are redesigning the sample cell to allow multiple passes of the infrared laser through the sample which will increase the absorption sensitivity still further.

PAPERS/JOURNALS/PUBLICATIONS:

A manuscript describing the first results is currently in preparation.

LDRD FUNDING:

FY 1994	\$ 57,142
FY 1995 est.	59,600

State-Resolved Measurements of H₂ Photodesorption: Development of Laser Probes of H₂ for In-Situ Accelerator Measurements

M. G. White
and R. J. Beuhler

94-05

PROJECT DESCRIPTION:

The UV/VUV photodesorption of H₂ and other background gases found in accelerator vacuum systems is being studied by state-selective laser methods. These measurements are pertinent to synchrotron radiation induced desorption which plays a major role in determining the beam chamber vacuum and lifetime of high energy electron/proton storage rings.

TECHNICAL PROGRESS AND RESULTS - Fiscal Year 1994:

Purpose: The purpose of this project is to explore the dynamics of H₂ production by photodesorption from cryogenically cooled metal surfaces such as those found in superconducting magnets of the next generation particle accelerators. Photodesorbed gases, especially H₂, are expected to play a major role in determining the lifetime of stored beams in large accelerators where synchrotron radiation strikes the surfaces of the cooled (4.2 K) superconducting bend magnets. We propose to study the desorption of H₂ and other small molecules (CO, N₂, and CO₂) by state-selective laser techniques which can provide information on the desorption mechanism and energy distributions of the desorbed molecules. The outcome of this work should result in a

detailed characterization of the H₂ photodesorption process as well as a prototype design for in-situ laser measurements in accelerator vacuum systems.

Approach: State-selective laser techniques and mass spectrometry are used to measure the translational energy as well as the internal state distributions of small molecules desorbed from cryogenically cooled metal surfaces. Our approach makes use of coherent vacuum ultraviolet (VUV) radiation to photoexcite desorbed molecules with high sensitivity ($\leq 10^7$ molecules per quantum state) and selectivity (0.7 cm⁻¹ bandwidth) and detect single rovibronic levels by resonant multiphoton ionization (REMPI). From a combination of time-of-flight arrival distributions and rovibrational spectra of intermediate electronic states, it will be possible to determine the velocity and state distributions of molecules photodesorbed by a second UV/VUV laser. A number of common beam tube materials such as Cu, Al and stainless steel, will be used to evaluate the sensitivity of the desorption properties on metal substrate. The VUV REMPI method will focus primarily on H₂ desorption but will be extended to detect CO, N₂ and CO₂ which includes the most important desorption gases in UHV vacuum systems. These studies will also be useful for evaluating the utility of laser-based *in situ* measurements of H₂ densities in accelerator environments.

Technical Progress and Results: A new, ultra high vacuum (UHV) apparatus has been constructed for state-selective detection of molecules desorbed from cryogenically cooled metal surfaces. The apparatus includes linear time-of-flight (TOF) mass spectrometer for species identification and translational energy distributions, a triply-differentially pumped laser VUV radiation source for state-selective excitation of desorbed molecules, and a fully

rotatable, LHe cooled sample holder for mounting of metal crystals and angularly dependent desorption measurements. An excimer laser operating at 193 nm is used to induce electronically stimulated desorption while an IR laser operating at 1064 nm is employed for studies of laser-induced "thermal" desorption.

Initial measurements have focussed on the IR laser-induced "thermal" desorption of CO on low temperature copper surfaces. These experiments provide new information on desorption caused by rapid heating of the metal surface by photon irradiation which is always a component of the photodesorption process. Sample results are given in the

accompanying figure which shows the extracted ground rotational state distributions (J'') of desorbed CO from which we derive a rotation "temperature" by simulation with a Boltzman fit. The results indicate near equilibration of the temperature between the surface atoms and desorbing molecules, suggesting long residence time on the surface before desorption. Translation energy measurements are currently underway and extension to H_2 (D_2) is planned in the next few months.

LDRD FUNDING:

FY 1994	\$ 68,871
FY 1995 est.	77,300

Siberian Snake Prototype Development For RHIC

M.A. Harrison

94-06

PROJECT DESCRIPTION:

A helical dipole magnet is one in which the dipole field, rather than remaining vertical, rotates uniformly along the length of the magnet. Such magnets are required to do spin physics at RHIC. The parameters required include the following: $B \sim 4T$, aperture = 100 mm, low current operation ($< 500A$) to minimize heat leak through many necessarily separated leads, and a pitch in the helix of approximately 180 degrees in one meter. There is no published record that such magnets have been built in the past; this is not surprising since they serve no useful purpose in normal accelerator or beam transport optics.

TECHNICAL PROGRESS AND RESULTS - Fiscal Year 1994:

Purpose: The purpose of this R&D program is to demonstrate the feasibility of implementing polarized protons in the RHIC accelerator by constructing a prototype Siberian Snake module.

Approach: Design Principles: Various possible ways to build a helical magnet were considered without any particular method standing out as obviously superior. The usual rules of engagement for the building of superconducting magnets still apply: that high forces be contained, that superconductor motion (particularly stick/slip motion) be minimized, that energy be safely extracted

from the magnet at quench, that the ends of the magnet be restrained, that cooling be adequate for the operational conditions, etc. The design that is presently being developed borrows some concepts developed in the SSC and RHIC magnet programs, in particular the coils of the RHIC sextupole magnet and the assembly methods used for RHIC correctors. However, the critical question of how to build a spiral coil uses a new concept: grooves milled into a thick-walled aluminum cylinder to give a $\cos(\theta)$ current distribution when the grooves are filled with conductor. Unfortunately, this approach mandates that there will be considerable labor required in building model magnets because the many turns forming the coils will have to be wound by hand. The positive side of this approach is that models can be built without a large tooling expense. If this design goes into production, then some relatively straightforward tooling can be built to reduce the labor required to acceptable levels.

Technical Progress and Results: It was found that the superconductor wire being used in the RHIC corrector program could be used for the helical magnet design if it is wound into a 7-strand cable. This conductor, nearly one mm in diameter, would require 382A to produce a 4T field in the present design. By using this existing wire, the need to develop a new superconductor is circumvented. Using a cable in the magnet is preferable to using a single wire: if a break in a wire should occur, the magnet would very likely still operate. The required cable has been manufactured in sufficient quantity for a first model of the present coil design. The required Kapton insulation was also wrapped onto the cable in the manufacturing operation.

As stated, this small diameter cable will be hand-wound under slight tension in an ordered pattern into slots milled into an aluminum cylinder. A piece of prepreg fiberglass cloth will be placed between each layer of wires in the slots. Pins at the ends will allow the winding to

proceed without wires bunching together. When all the turns have been wound onto the cylinder, they will be compacted with fiberglass tape wound around the outside under tension, and then the entire assembly will be placed into an oven to cure, thereby forming a series of current blocks around the cylinder in which each wire is firmly supported in a fiberglass/epoxy matrix. This design for supporting the cable turns is analogous to that developed for the wire turns in the RHIC sextupole magnet. The ends will be filled with a mineral-loaded epoxy and cured in a follow-on operation, a technique used in the SSC program for adding strength and rigidity to coil ends.

Three of these cylinders, concentric with one another, are required to give the required field of 4T. These three cylinders will be mounted into an iron yoke using a support scheme as is being used for the RHIC corrector magnets. A helium containment shell is then welded in place around the yoke, serving also as the magnet support structure. From this point, the design is similar to that of the arc magnets for RHIC and all the same concepts and methods will be used as appropriate.

It is estimated that the helical magnet operating at 4.2K will have a margin in field of 16% above 4T. This is too slender a margin for an untested design such as this, so an operating field of 3.5T is recommended for this design, giving in the case a margin of 33%. Undesired harmonics were easily minimized in this design by adjusting the thickness of the walls between current blocks, a procedure analogous to adjusting coil wedges in a conventional magnet.

Status: The parameters for a first model have been established. Superconducting cable wrapped with kapton has been delivered to BNL and tested. A suitable magnetic design, including end design, has been made. The engineering design for one of the required three cylinders has been completed, material has been procured, and the cylinder has been manufactured in the BNL

shops. A technician has been assigned to set up a workspace and to begin winding superconductor into the grooves of the cylinder.

Future Work: Lab: It is most important to establish the feasibility of winding the coils. This will be the focus of effort in the early part of the next fiscal year. When a single current block has been successfully built, the coil will be tested cryogenically to see if short sample in the superconductor is achieved. Following this, a complete cylinder will be wound and then tested. The design will be modified as indicated by the test results from each of the partial coil trials. Simple tooling will be fabricated as required. Upon successful completion of a single cylinder, the other cylinders will be fabricated and a completed cold mass including yoke will be fabricated and tested.

Design: The engineering design of the balance of the magnet will be continued. Quench propagation and quench temperatures in the magnet will be modeled. It will be necessary to establish methods for measuring the fields in the magnet, in collaboration with the requirements of accelerator physicists needs for modeling beam behavior in this type of magnet. An effort will be made to work with industry in order to make a production design.

FOLLOW-ON FUNDING:

Negotiations for full project funding are underway with the Japanese research institute at RIKEN.

LDRD FUNDING:

FY 1994	\$132,139
FY 1995 est.	134,000

Synthesis and Characterization of Novel Microporous Solids

J. A. Hriljac

94-09

PROJECT DESCRIPTION:

Synthetic routes to new large-pore microporous solids are being developed and these, and related materials, are being studied using powder and microcrystal diffraction techniques.

TECHNICAL PROGRESS AND RESULTS - Fiscal Year 1994:

Purpose: Crystalline large-pore molecular sieves are important materials for a variety of industrial applications in traditional fields such as catalysis and sorption, and are also needed as new hosts for hybrid inclusion compounds (such as those formed with semiconductor, organo-metallic, or organic molecules) with novel electronic or optical properties. The rational synthesis of new frame-works presents a tremendous scientific challenge because of a limited knowledge of the crystallization mechanism(s) and the metastable nature of molecular sieves. The work in this program is aimed at increasing the understanding of the formation of these compounds as well as synthesizing and characterizing new materials. The work relies heavily on the ability to perform crystallographic work on powders or small single crystals, and includes the development of the appropriate techniques, instrumenta-tion, analysis methods, and their implementation at the National Synchrotron Light Source (NSLS) and High Flux Beam Reactor (HFBR).

Approach: Two related studies are being undertaken to elucidate the chemical factors which produce large-pore molecular sieves. The first of these is the role of templates. It is well known that one can influence the products formed from a reactive gel via 'structure directing' or 'templating' by the addition of appropriate cations. The nature of this process and the details of the gel chemistry are not well understood, and further work is clearly needed if the rational design of new molecular sieves is going to be achieved. To elucidate the template-solid relationship a series of related molecular sieves will be prepared and the crystal structures determined before any calcinations or treatments. Attempts will then be made to correlate the template shape and siting with the pore structure. The second study will explore the synthesis of materials with unusual chemical compositions, particularly borates. The approach is based on the postulate that in the aluminosilicate zeolites there is a limit on the possible topologies that is imposed by the strictly tetrahedral coordination of silicon, the connectivity of the tetrahedra only through corners, and a rather narrow range of observed Si(Al)-O-Si(Al) angles. These constraints are relaxed in the aluminosilicates and gallophosphates and one finds not only aluminum in 5- and 6-coordinate sites but also a greater number of well defined large-pore sieves. Micro-porous borates should offer a rich diversity of topologies, as boron is often three- or four-coordinate and forms a variety of ring and cage compounds.

The crystallographic work includes the continuing development of techniques for solving structures *ab initio* from high resolution powder diffraction data and

refining the structural models against synchrotron x-ray and neutron data. It also includes the development and implementation of the use of imaging plates for the collection of diffraction data from micron-sized single crystals.

Technical Progress and Results: (a) The supposedly well defined aluminophosphate AlPO-5 was chosen for the template crystallo-graphic studies as it has a reasonably simple structure and can be crystallized using a large variety of organic cations. A large batch of small (ca. 40 μm) single crystals was produced using triethylamine as the template. These are being studied at the NSLS beam line X7B in collaboration with Jon Hanson in the Chemistry Department at Brookhaven National Laboratory (BNL). Step scanning data have been collected on both the as-prepared and empty (calcined) frameworks, and attempts made to refine the crystal structures. More accurate and complete data sets are needed to resolve some subtle problems and obtain a more precise picture of the template. Three sets of imaging plate data have been collected and the data are being reduced and averaged as described in section (e) below.

(b) Exploratory syntheses of microporous borophosphates have begun. A series of reactions has led to the isolation of two solid phases. Preliminary inspection of the x-ray powder diffraction patterns suggests they are new materials, and high resolution synchrotron data have now been collected to determine the crystal structures. Further analysis is needed to determine if these are indeed novel phases and, if so, if they are microporous.

(c) In a continuing collaboration with C. C. Torardi at DuPont Central Research the

structure of a novel bismuth molybdate, $\text{BiMo}_2\text{O}_7\cdot\text{OH}\cdot 2\text{H}_2\text{O}$, was determined from high resolution synchrotron data. This material is prepared under mild hydrothermal conditions and is related to a family of selective oxidation catalysts. Initial screening at DuPont indicated that it is not thermally stable and therefore not a catalyst itself. A knowledge of the structure is important not only for characterization purposes but also to determine if catalytic activity is expected if a stable derivative could be produced. The x-ray data were sufficient to describe the metal oxide part of the structure and the layered nature of the material, but could not be used to elucidate the hydrogen bonding network that holds the layers together. A deuterated analog has been prepared and powder neutron diffraction data were collected at the new H1A spectrometer at the HFBR. Rietveld analysis has produced two possible models for the deuterium positions and further analysis will determine which of these is correct.

(d) Another ongoing collaboration is with J. B. Parise at the State University of New York at Stony Brook, investigating the crystal structures of hydrothermally synthesized tin and antimony sulfides.

This work is aimed at producing microporous sulfides. The structures of those materials that cannot be produced as single crystals are being studied at the NSLS using *ab initio* powder techniques. One of these phases is a metastable intermediate that is produced when pyrrolidine is used as a template, and a knowledge of the structure is needed for a better understanding of the crystallization mechanism. The current model appears to

be correct but probably incomplete, and gives a formula of Sn_3S_7 pyrrolidine. Future work will explore the possibilities of lower symmetry space groups and should lead to a full description of the structure and its relationship to the more stable solid obtained upon further heating.

(e) The use of imaging plates for the collection of single crystal rotation data is being undertaken in collaboration with Jon Hanson of the Chemistry Department at beamline X7B. The experimental details needed to quickly produce images with good signal-to-noise have been worked out. The software developed by P. Coppens and co-workers at SUNY-Buffalo (IPMS, HIPPO) for data analysis has been implemented and tested. The first complete data set on a known material has been produced and the structural model accurately refined including thermal parameters to an R-value of ca. 2%. The compound was a sodium potassium silicate crystal of approximate dimensions $70 \times 70 \times 70 \mu\text{m}$ and a complete range of rotation data was collected in 1 hour (the actual exposure time was ca. 6 minutes).

PAPERS/JOURNALS/ PUBLICATIONS:

A manuscript entitled "A description of the hydrogen bonding in the novel layered oxide $\text{BiMo}_2\text{O}_7 \cdot 2\text{D}_2\text{O}$ as determined from a high resolution neutron powder diffraction study" is being prepared and will be submitted in the near future.

LDRD FUNDING:

FY 1994	\$113,862
FY 1995 est.	113,000

Ozone Depletion, Chemistry, and Physics of Stratospheric Aerosols

Dan G. Imre and
Ignatius N. Tang

94-10

PROJECT DESCRIPTION:

The goal of this proposal is to initiate a new research program to study the physics and chemistry of stratospheric aerosols. Many atmospheric processes are thought to proceed via heterogeneous reactions between gas phase molecules and aerosol particles. Aerosols have been implicated as major participants in the formation of the Polar Ozone hole. Furthermore, by converting reactive Nitrogen to Nitric Acid, they also play a very important role in maintaining the Stratospheric Nitrogen balance. Since all atmospheric reactions involve reactive Nitrogen, aerosols therefore indirectly also control the entire atmospheric chemical balance.

We propose to construct a novel experimental setup in which the physics and chemistry of single levitated aerosol particles under stratospheric conditions will be studied. Using Mie scattering as well as Laser Raman Spectroscopy we will observe and map exact points of phase transition. We will be able to generate and study states of super saturation and super cooling which are unique to aerosols. Furthermore, Raman spectroscopy will enable us to document the various crystal structures and compositions of frozen particles, as well as to follow in detail heterogeneous chemical processes. This will be the first set up of this kind, providing the first glimpse into the chemistry of single levitated aerosol particles.

TECHNICAL PROGRESS AND RESULTS - Fiscal Year 1994:

Purpose: Few recent scientific observations have captured our attention as did the discovery of the Ozone hole in 1985. It sparked an enormous amount of research and controversy. The proposed research program addresses one of the crucial steps in the mechanism believed to lead to the destruction of the Ozone layer. It will develop a novel experimental approach to investigate the physics and chemistry of aerosols under stratospheric conditions. Participants in the most recent IPCC WGI/OZONE Assessment Workshop (May 1993), identified an urgent need to have information concerning basic thermodynamic properties of stratospheric aerosols, whose importance is not limited to the Ozone issue alone, but extends to many climatological phenomena. Surprisingly to date, not a single laboratory study on stratospheric aerosols is available. Even the most fundamental of properties - the physical state of stratospheric particles can not be unambiguously predicted.

The discovery of the Antarctic Ozone hole in 1985 by Farman et al. came as a complete surprise; every available study to that date predicted Ozone depletion at high altitudes (~40 km) and at mid latitude². The observation could not possibly be explained by considering gas phase chemistry alone. In 1987 a new mechanism involving heterogeneous chemistry catalyzed by stratospheric cloud particles was proposed³, and has served as the prevalent explanation for polar Ozone depletion ever since. The extreme low temperatures of the polar winters are known to result in the formation of Polar Stratospheric Clouds (PSCs) which are ice, or Nitric Acid / ice particles. The surface of these particles was hypothesized to serve as a

catalyst for the conversion of inactive Chlorine-HCl and ClONO₂, to its reactive forms that destroy Ozone. The supporting evidence for this mechanism is based on laboratory experiments⁴⁻¹⁸ in which, stratospheric aerosols are simulated by thin ice films, with not even an attempt at surface characterization. To date there has not been a single case where the proposed Ozone depletion chemistry has been shown to work on actual aerosols.

Approach: Developing a new experimental set up that will trap a single isolated aerosol and keep it under stratospheric conditions (temperature as low as 180 K and pressure of 50 torr). This set up will enable the control, and cycling of temperature, relative humidity and gas phase constituents much as they occur in the ambient stratosphere. This experimental setup will combine light scattering with Raman spectroscopy of single levitated particles. Precise thermodynamic properties of super cooled and super saturated droplets will be determined using Mie scattering. Chemical properties will be studied using laser Raman spectroscopy, a highly sensitive tool for distinguishing one chemical state from another. For example we should, for the first time, be able to determine the exact crystalline states of ice clouds.

Technical Progress and Results: A six window low temperature single particle cell has been designed and constructed. Figure 1 is a diagram that illustrates the major components. The cell is LN₂ cooled, heat exchange with the electrostatic trap is achieved using veritable number of heat conductors. The cell is configured such that it floats at 170 K. Temperature is controlled using two 50 ohm heaters. Temperature is monitored using diodes

at three locations to insure even heating. Temperature can be scanned or held fixed using a feedback loop. An ultrahigh vacuum system incorporating the cryostat was constructed. The entire system is designed to allow gas flow. The mie scattering laser was mounted and a data collection system was constructed, with the entire system being computer controlled. An initial room temperature calibration run using NaCl has been completed.

In parallel with the construction of the new instrument, study of room temperature model systems has been completed. Experiments on bisulfate particles has revealed a new crystalline phase that has never been previously observed. LiClO₄ has provided a first example of solid/solid phase transitions of the kind expected to occur in stratospheric particles. Normal bulk solid/ solid phase transitions in Sr(NO₃)₂, and Na₂SO₄ have been found to be completely missing. These experiments demonstrate that the bulk data which has thus far been used to predict particle behavior is insufficient, and in many cases misleading.

PAPERS/JOURNALS/PUBLICATIONS:

A paper summarizing our findings on model systems is in preparation.

"Metastable Physical States of Hygroscopic Aerosols", October 1994, in preparation for *Science*.

LDRD FUNDING:

FY 1994	\$ 99,533
FY 1995 est.	105,000

Figure 1

RF Sources for Linear Colliders

R. Palmer
and R. Fernow

94-21

PROJECT DESCRIPTION:

The Cluster Klystron is being developed as an 11.4 GHz pulsed RF power source for future linear colliders. One of the major goals of the project is to achieve high efficiency through the use of multiple, low current beams, through the use of a modulated anode, and by not requiring RF pulse compression. The proposed experiment involves the construction of three magnetron injection guns (MIGs) enclosed in a common solenoidal magnetic field. The work is currently being done in collaboration with SLAC, Varian Corp., and MIT.

TECHNICAL PROGRESS AND RESULTS - Fiscal Year 1994

Purpose: Deeper understanding of the fundamental principles of particle physics will require the construction of new accelerators capable of reaching the TeV energy scale. Linear electron-positron colliders, where the beams from two accelerators collide head on, are considered by many to offer the best hope of reaching this new energy regime. The current SLAC electron linac is powered by klystrons that operate at a frequency of 2.86 GHz. Scaling studies have indicated that it should be cheaper to build a linac that operates at a higher frequency. The source must be capable of producing power outputs around 100 MW, with a pulse length around 1 microsecond, and with the highest electrical efficiency possible.

For a given accelerating voltage in a klystron-type source there is an inverse relation between the efficiency and the current. In a normal klystron, however, low current would imply a low power output. The basic idea of the cluster klystron concept is to get both high power and good efficiency by using many low current beams in parallel. The cluster klystron idea of Palmer, Herrmannsfeldt, and Eppley proposed to use compact magnetron injection guns as the electron sources and to put the whole assembly inside one common solenoidal field.

If the cluster klystron project is successful in simultaneously achieving all the required physics parameters, it could be a leading candidate for the power source for a future linear collider. However, another important question for any potential source is economics. This includes both the cost of constructing the source and the cost of operating it. There is reason for hope with this concept in both regards. The cluster klystron uses a mod-anode, so it does not require the expensive pulsed modulator used with conventional klystrons. In addition, the mod-anode determines the length of the RF pulse directly, so expensive pulse compression systems are not required. The expected high efficiency should result in the lowest possible operating costs.

Approach: Work on the cluster klystron project has been divided into four phases. The first is to build and test the operation of the high current density, magnetron type guns at potentials up to 100 kV. The initial arrangement will use one beam together with an electron beam diagnostics chamber and a single 4-cell klystron cavity. This will allow us to test many of the fundamental properties of the ultimate device including stability of the MIG and switching the current with the mod-anode. Special runs will investigate

acceleration of the beam off the symmetry axis of the electric and magnetic fields.

The second phase will test the clustering concept by using three beams with three 4-cell klystron cavities. The third phase would involve increasing the dc potential up to 400 kV and lengthening the solenoid to accommodate the 16-cell klystron cavity. This would provide the first tests of achieving high efficiency. The fourth phase will test a complete cluster of three beams with three high efficiency klystron cavities.

Technical Progress and Results: Additional tests were made using the solenoid magnet in our experimental area in building 901. Magnetic shims were inserted inside the solenoid to demonstrate that we can produce transverse magnetic fields on the axis of the solenoid. This technique will allow us to simulate the effect of off axis operation during Phase 1 of the project. The magnetic field profile was measured using a Hall probe. Good agreement was found with computer predictions.

A mock-up was made of the long stock that holds the cathode of the device. Accelerometer measurements were performed and indicated that vibrations of the stock should have an amplitude of less than 1 micron. Therefore, vibrations should not be a problem for gun operation.

Detailed theoretical and computer simulation studies were done of the electron beam behavior in the acceleration gap and drift tube regions of the device. These included 3-dimensional simulations using the computer code MAFIA. It was shown that azimuthal motions of the electron beam are small, so that they should not cause difficulties in operation.

Additional progress was made in refining the engineering details for the phase 1 experiments. General layouts have been prepared for all the required mechanical assemblies. Detailed shop drawings were made for the vacuum system and high voltage test stand. All the parts for the vacuum system have been either purchased or built in the machine shops.

Detailed computer designs have been made of a set of diagnostic RF cavities for initial testing, as well as a set of high efficiency cavities for power generation. Reasonable agreement for the bunching and efficiency was found among three computer codes and an analytic calculation.

David Yu Associates, with whom we are collaborating, submitted a proposal for a phase I STTR grant to continue work on RF output couplers and combiners. These devices will be required in phase 4 of our project to combine the power from the three beams in the cluster.

PAPERS/JOURNALS/PUBLICATIONS:

Three presentations were made at the International Workshop on Pulsed RF Power Sources for Linear Colliders at Montauk, New York in October, 1994.

FOLLOW-ON FUNDING:

A request for project funding for FY 1995 and beyond has been submitted to the Department of Energy.

LDRD FUNDING:

FY 1994	\$ 97,788
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Resonance Raman Detection of Volatile Organic Chemicals (VOCs)

R. E. Barletta

94-26

PROJECT DESCRIPTION:

Resonance enhancement of the Raman cross sections of volatile organic chemicals (VOCs) is being studied to provide a data base to allow for the development of *in situ* monitoring of these chemicals in the environment.

TECHNICAL PROGRESS AND RESULTS - Fiscal Year 1994:

Purpose: A continuing issue in the on-going site remediation projects at various locations throughout the Department of Energy complex is the ability of detect volatile organic chemicals (VOCs) at trace concentrations (in the range of parts per billion). Hence, there is a need for technology to analyze for the presence of these chemicals *in situ* at levels of regulatory concern. These sensors are required to be field portable and capable of providing near real-time data, i.e. <10 minutes per sample.

Molecular spectroscopy and, in particular, Raman spectroscopy has the potential to be the basis of an analytical method for such measurements. Under non-resonant conditions, however, the magnitude of the cross section is too small to allow for detection at the ppb level. Large increases in the Raman cross section (factors of 10^5 to 10^6) occur as the excitation frequency nears and overlaps an electronic transition, and it is this increase

which will allow for trace detection of VOCs.

The application of the technology to the analysis of soils and other field situations should be rather straightforward once an estimate of the resonance enhanced cross section is available. For many compounds of interest, such measurements have not been made since the electronic transitions of compounds such as CCl_4 and CHCl_3 are in a region which has only recently been accessible with UV-laser sources. Resonance enhanced cross sections for one or more VOCs of interest to site remediation need to be measured. Once these measurements have been made, it will be possible to make sensitivity calculations for prototype *in situ* Raman-based systems.

Approach: To measure the resonance enhancement of the Raman cross section, it was necessary to fabricate the appropriate cell to allow gas phase Raman spectra to be taken. Once this was completed Raman spectra of selected VOCs along with reference chemicals could be obtained. The intensity of the Raman bands of the VOCs was compared with that of a reference gas whose absolute cross section is known and which is not resonantly enhanced in the region of the measurements. The data was further compared to literature values of relative Raman cross sections in regions far from resonance. In this manner, the magnitude of the resonance enhancement was calculated and the resonance Raman cross section derived.

Technical Progress and Results: (a) A Raman cell capable of being used for either liquid or gas phase measurements was designed and fabricated. The design of the

cell had to address the issues of laser damage to the entrance window while minimizing the absorption of both incident and scattered radiation. A schematic of the cell is shown in Figure 1.

(b) Three VOCs were chosen for study, CHCl_3 , CCl_4 and C_2HCl_3 . Each of these chemicals are of interest from a site remediation standpoint and each has an electronic absorption which overlaps the excitation wave-length of the KrF laser being used (248 nm). In addition, two reference gases were chosen N_2 and H_2 .

(c) For two of the VOCs, CHCl_3 and C_2HCl_3 , photochemical decomposition as well as the high extinction coefficient of the parent molecule electronic absorption at the frequency of the Raman scattered light precluded the observation of a Raman signal. Re-design of the cell and optical system to correct for this absorption was outside the level of effort for this LDRD. For N_2 , it was only possible to observe the Raman-active vibrational Q-branch under optical conditions which precluded comparison with data taken in the Raman cell shown in Figure 1. Thus, data which could be used for cross section measurement was only obtained for the VOC CCl_4 and the reference gas H_2 .

(d) Like the other VOCs studied, carbon tetrachloride also exhibited photodecomposition. In the case of CCl_4 , however, the Raman signal from the ν_1 band ($\Delta\text{cm}^{-1} = 459$) was sufficiently strong that the signal could be observed with a single laser pulse. Further, the frequency of the Raman shifted light occurred in a region in which there was minimal absorption by the CCl_4 vapor over the distance between the measurement region and the optical fiber which collected the scattered radiation.

(e) The Raman spectrum of CCl_4 was obtained at a variety of gas pressures and incident laser energies. A typical spectrum is shown in Figure 2. Of the features evident in this spectrum, only the band at 459 cm^{-1} can be unambiguously assigned to a Raman band of carbon tetrachloride. The other fundamentals were not observed, nor were bands due to obvious decomposition products such as Cl_2 , Cl_3 or C_2Cl_6 . This is not surprising since the ν_1 band should show the largest resonance enhancement given that the lowest electronic absorption is a state in which the absorption results in the dissociation of the molecule.

It was found during the course of these studies that the intensity of the 459 cm^{-1} band is a linear function of both gas pressure and laser energy.

(f) Based on the intensity measurements for CCl_4 as well as measurements on the Q-branch of H_2 , the resonance enhancement for CCl_4 was determined to be $2.4 \pm 1.1 \times 10^4$ at 248 nm. Using this value along with literature data for the Raman cross section measured under non-resonant conditions an estimate of the sensitivity for the detection of CCl_4 is estimated to be ~ 40 ppb with a signal to noise of about 10. In addition, it was determined that it should be possible to detect atmospheric CCl_4 in concentrations in the sub-ppm regime over short ranges ($<0.5\text{ km}$).

PAPERS/JOURNALS/PUBLICATIONS:

A manuscript entitled "Resonance Raman Spectrum of Carbon Tetrachloride" has been submitted to Applied Spectroscopy for publication. In addition, a report entitled "Resonance Raman Spectroscopy of Volatile Organics - Carbon Tetrachloride", BNL-60755, has been published.

FOLLOW-ON FUNDING:

Initial contact has been made with potential sponsors within DOE for funding under DOE's waste management activities. In addition, a joint program with a private company to develop a system for monitoring VOC contamination at a DOD site is being pursued.

LDRD FUNDING:

FY 1994

\$ 100,338

Figure 1. Schematic of Raman cell. a. lens; b. quartz window; c. fiber optic to detector; d. measurement region; e. rectangular quartz tubing (1.0 cm x 0.5 cm); f. greaseless stopcock; g. 1/4 Pyrex tube for connection to the gas handling system; h. excimer laser beam.

Figure 2. Raman spectrum of CCl_4 with KrF laser excitation (248 nm). The CCl_4 pressure was 66 Torr and the laser energy was 68 mJ. The data was taken with a single laser pulse.

Understanding the Molecular Basis for the Synthesis of Plant Fatty Acids Possessing Unusual Double Bond Positions

J. Shanklin

94-31

Fatty acid unsaturation is an important component of the nutritional and industrial quality of seed oils of crop plants. One factor influencing the properties of seed oils is the degree of unsaturation, or numbers of double bonds, of the constituent fatty acids. Also, important is the position of double bonds within the carbon chain of fatty acids. For example, oleic acid (18:1 Δ^9), the most common monounsaturated fatty acid present in seed oils, is an 18 carbon fatty acid with a double bond at the ninth carbon atom from the carboxyl end of the molecule. This fatty acid has a melting point of approximately 14°C, and, as such, oils rich in oleic acid are liquids at room temperature. By simply moving the double bond to the sixth carbon atom, the resulting fatty acid, petroselinic acid (18:1 Δ^6), has a melting point twice that of oleic acid. In addition, petroselinic acid, because of the position of its double bond, can serve as an industrial precursor of surfactants and nylon monomers.

Unsaturation in seed oils is the result of the activity of fatty acid desaturases, the enzymes that catalyze the insertion of double bonds into fatty acids. These enzymes typically have strict specificity for the chain length of the fatty acid on which they function and for the position at which they introduce double bonds into fatty acids. The ability to alter the chain length and double bond-positional specificities of desaturases

could lead to a means of producing seed oils with new physical and industrial properties, as illustrated in the example above. To do this, requires an understanding of structural features of desaturases that influence how these enzymes recognize and interact with fatty acid substrates. Available to address this question are cDNAs encoding structurally related but functionally variant acyl-acyl carrier protein (ACP) desaturases: Δ^9 -stearoyl (18:0)-ACP desaturase, Δ^4 -palmitoyl (16:0)-ACP desaturase, and Δ^6 -16:0-ACP desaturase. These higher plant enzymes share a high degree (> 70%) of primary structural similarity and presumably function through the same catalytic mechanism. Despite this, these desaturases display substrate preference for fatty acids of different chain lengths (16:0 vs. 18:0) and insert double bonds at different positions within acyl chains (Δ^4 , Δ^6 , or Δ^9). The research described here is aimed at comparing the active site structures of members of this family of enzymes. Information gained from this research may allow for the design of fatty acid desaturases that are capable of synthesizing industrially-useful isomers of unsaturated fatty acids in crop plants.

TECHNICAL PROGRESS AND RESULTS - Fiscal Year 1994:

Purpose: The goal of this work is to understand the structural basis through which fatty acid desaturases of higher plants recognize the chain lengths of substrates and position the placement of double bonds within acyl chains. This will be accomplished through comparative biochemical studies involving higher plant acyl-acyl carrier protein (ACP) desaturases. We have chosen to work with plant acyl-ACP desaturases because these enzymes display soluble activity and are thus more

amenable to biochemical characterization than are the membrane-bound desaturases of mammalian and yeast systems. In addition, the availability of several functionally variant acyl-ACP desaturases of similar primary structure (as described above) offers a unique opportunity to determine structural parameters associated with interactions of desaturases with their fatty acid substrates. It is anticipated that results from mutagenesis and X-crystallographic studies outlined below will allow for the engineering of desaturases capable of introducing double bonds into different positions of fatty acids of a range of carbon chain lengths.

Approach: We currently have available cDNAs for Δ^9 -stearoyl (18:0)-ACP desaturase, Δ^4 -palmitoyl (16:0)-ACP desaturase, and Δ^6 -16:0-ACP desaturase. These cDNAs are being used as tools for mutagenesis studies. By "swapping" portions of cDNAs of one type of acyl-ACP desaturase with that of another, attempts are being made to determine what domains of these desaturases account for differences in their abilities to position double bond insertion in acyl chains and to recognize the chain lengths of fatty acid substrates. Following mutagenesis, cDNAs are expressed in *Escherichia coli* using the bacteriophage T7 expression system. The resulting desaturases are assayed *in vitro* using radiolabeled substrates to determine whether a given mutagenesis step has, for example, lead to the conversion of a Δ^9 -18:0-ACP desaturase to a Δ^6 -16:0-ACP desaturase.

To facilitate screening of mutants, we are developing a system that will allow acyl-ACP desaturases to function *in vivo* in *E. coli*. Such a system will allow one to assess whether a mutation has lead to an alteration in the chain-length or double-bond positional specificity of a desaturase by

simply monitoring the fatty acid composition of recombinant *E. coli* by gas chromatography. *E. coli* and plants share a very similar fatty acid biosynthetic system. However, *E. coli* apparently lacks the proper forms of the oxidation-reduction system (namely, ferredoxin and ferredoxin-NADP reductase) required for acyl-ACP desaturase activity. We are therefore engineering *E. coli* to co-express mutagenized cDNAs for acyl-ACP desaturases along with cDNAs for plant ferredoxin and ferredoxin-NADP reductase.

To more directly determine how structural differences result in functional variations in acyl-ACP desaturases, the three-dimensional conformations of Δ^9 -18:0-ACP desaturase and Δ^6 -16:0-ACP desaturase will be compared. X-ray crystallographic analysis of the Δ^9 -18:0-ACP desaturase is nearing completion. These studies are being conducted by our lab in collaboration with the group of Branden, Uppsala, Sweden. To complement this work, we are attempting to over-express the Δ^6 -16:0-ACP desaturase in bacteria and purify large amounts of this enzyme for crystallographic study. By comparing the three-dimensional structures of Δ^9 -18:0-ACP desaturase and Δ^6 -16:0-ACP desaturase, it will be possible to more finely tune the mutagenesis studies described above. It is expected that through the combined results of mutagenesis and x-ray crystallography it will be possible to precisely identify how acyl-ACP desaturases that share very similar primary structures differ in their interactions with fatty acid substrates.

The above structural studies would be aided by the isolation of additional cDNAs for variant acyl-ACP desaturases. Likely sources of such desaturases are plant species whose seed oils contain large amounts of

unusual monounsaturated fatty acids. One such plant is milkweed whose seed oil is enriched in palmitoleic acid (16:1 Δ^9) and *cis*-vaccenic acid (18:1 Δ^{11}). Experiments are being conducted to determine whether these fatty acids result from the activity of a Δ^9 -16:0-ACP desaturase. Comparative structural studies between this enzyme and the Δ^9 -18:0-ACP desaturase may provide a means of specifically characterizing the fatty acid chain-length specificity of acyl-ACP desaturases.

Technical Progress and Results: When the primary structures of Δ^9 -18:0-ACP desaturase, Δ^4 -16:0-ACP desaturase, and Δ^6 -16:0-ACP desaturase are compared, the most significant difference between these enzymes is observed near their N-termini. In this region, the Δ^4 -16:0-ACP desaturase, and Δ^6 -16:0-ACP desaturase lack 15 and 6 amino acids, respectively, relative to the Δ^9 -18:0-ACP desaturase. In mutagenesis studies, this region has been "swapped" between the Δ^6 -16:0-ACP desaturase and Δ^9 -18:0-ACP desaturase. In the case of the Δ^9 -18:0-ACP desaturase, this change had no effect upon the fatty acid chain length and double bond positional specificity of the enzyme. In addition, complete removal of this region did not alter the functional properties of the Δ^9 -18:0-ACP desaturase. These results suggest that variability in the N-termini of acyl-ACP desaturases does not account for the differences in the activity of these enzymes. Further mutagenesis studies, however, have identified a region of approximately 50 amino acids in the middle of acyl-ACP desaturases that may define how these enzymes interact with fatty acid substrates.

As a first step in determining the three-dimensional structure of the Δ^6 -16:0-ACP desaturase, we are attempting to express high levels of this enzyme in *E. coli*. Initial efforts to express Δ^6 -16:0-ACP desaturase behind the T7 RNA polymerase promoter resulted in the production of only small amounts of recombinant protein. However, by lowering the temperature of expression to approximately 22°C, we have dramatically increased levels of Δ^6 -16:0-ACP desaturase production in *E. coli*. In addition, a large portion of the recombinant protein is recovered in a soluble form. The expressed protein has subsequently been enriched to > 85% purity by anion-exchange and hydroxyapatite chromatography.

Studies have been initiated to determine the biosynthetic origin of the unusual fatty acids palmitoleic acid (16:1 Δ^9) and *cis*-vaccenic acid (18:1 Δ^{11}) in seed oil of milkweed. A cDNA library has been constructed from poly A+ RNA of this tissue. From this library, a cDNA has been identified that encodes an enzyme that is diverged from that of known acyl-ACP desaturases. Screening of the library was accomplished using PCR with degenerate oligonucleotides corresponding to conserved regions of acyl-ACP desaturases. Studies are currently in progress to determine whether the desaturase encoded by the milkweed cDNA is associated with the biosynthesis of 16:1 Δ^9 and 18:1 Δ^{11} .

LDRD FUNDING:

FY 1994	\$64,808
FY 1995 est.	\$70,000

Structure Determination of Outer Surface Proteins of the Lyme Disease Spirochete

C. L. Lawson

94-32

PROJECT DESCRIPTION:

The structures of major antigenic outer surface proteins of the Lyme disease spirochete are being determined by x-ray crystallographic methods.

TECHNICAL PROGRESS AND RESULTS - Fiscal Year 1994:

Purpose: Our goal is to obtain detailed structural information needed for rational design of broad spectrum vaccines against the various strains of the *Borrelia* spirochete. OspA and OspB are major antigenic outer surface proteins of *Borrelia burgdorferi*; they share 53% amino-acid sequence identity, and so almost certainly share a common three-dimensional fold. The structure of at least one must be determined *de novo* because there is no significant sequence homology to other proteins of known structure.

Approach: OspA and OspB from strain B31 are readily purified from recombinant clones obtained from J. J. Dunn (Biology, BNL). Standard approaches to crystallization (i.e., vapor-diffusion experiments where temperature, protein concentration, precipitant, pH, and salt additive were varied) failed in preliminary trials. Therefore, the emphasis of our current work has been to expand our search for diffraction quality crystals over a much broader range of experimental conditions.

Our proposed strategy was to produce a range of protein specimens for crystal-

lization trials that are chemically and structurally distinct. Options we considered included: (1) varying the protein source strain, (2) preparing penicillin:outer surface protein complexes, (3) preparing Fab antibody fragment:outer surface protein complexes, (4) preparing the proteins with methylated lysine residues, (5) preparing the proteins with their natural lipidated amino-termini and (6) preparing proteolytic fragments. Although we have explored all of these options to some extent, we have chosen to focus on crystallization of Fab:OspA complexes. Structures of these complexes would provide us not only with coordinates for OspA, but would also allow us to define antigenic epitopes.

Technical Progress and Results: (a) Crystallization. We have grown crystals of an Fab:OspA complex using the Fab fragment of a mouse monoclonal antibody raised against whole spirochetes (α LS184.1). Thin, needle-shaped crystals were observed to form within one week at 4°C in 50 mM Tris-HCl pH 8.5, 80 mM MgCl₂, 4% (v/v) glycerol and 6-12% (w/v) polyethylene glycol 3750. Thicker crystals suitable for diffraction analysis with synchrotron radiation were subsequently obtained using microseeding methods. Gel electrophoresis experiments verified that the crystals are composed of the Fab fragment and OspA in a 1:1 molar ratio.

(b) Data collection. Diffraction images were collected in August 1994 at NSLS beamline X12C using an automated image plate detector system. The Fab/OspA crystals were mounted in sealed quartz capillary tubes and kept at 4°C. A high resolution diffraction pattern ($d_{\min} = 2.8 \text{ \AA}$) was observed from one of the larger crystals (Fig. 1), demonstrating that the crystals should be suitable for structure determination at near-atomic resolution. An 83% complete data set was collected on a smaller

single crystal with useful intensity data to 3.8 Å resolution. The crystal lattice was determined to be primitive orthorhombic with $a = 89.4$ Å, $b = 91.6$ Å, and $c = 103.1$ Å. We assume that there is one Fab/OspA complex per crystal asymmetric unit, with a solvent content of 56%. Since the Fab accounts for approximately two-thirds of the structure, we are currently attempting to use molecular replacement methods to find the orientation and position of the Fab in the crystal unit cell.

PAPERS/JOURNALS/PUBLICATIONS:

A manuscript entitled, "Cocrystallization of *Borrelia burgdorferi* Outer Surface Protein A with the Fab Fragment of a Neutralizing Mouse Antibody" is being prepared for submission to a peer-reviewed journal.

FOLLOW-ON FUNDING:

A National Institutes of Health proposal entitled "Vaccine Intervention for Lyme Borreliosis" was submitted in March 1994.

LDRD FUNDING:

FY 1994	\$ 76,787
FY 1995 est.	82,000

Fig. 1: Oscillation photograph ($\phi = 1.5^\circ$) of an Fab184.1/OspA complex crystal.

Low Mass, Low Cost, Multiwire Proportional Chambers for Muon Systems of Collider Experiments

V.A. Polychronakos

94-33

PROJECT DESCRIPTION:

This project deals with the development of economical, mass production techniques of Multiwire Proportional Chambers (MWPC) appropriate for use with Muon Systems covering large solid angles in modern Collider Experiments. These chambers would utilize modern lightweight composite materials and provide all functions needed in a Muon System, i.e. precision momentum measurement, transverse coordinate, timing, and trigger.

TECHNICAL PROGRESS AND RESULTS - Fiscal Year 1994

Purpose: Multiwire Proportional Chambers have been in use for many years. Their spatial resolution is generally limited by the anode wire spacing and is of the order of one millimeter. This is inadequate for precision measurements of high momentum particles in planned or future High Energy Hadron Colliders where resolutions at the 100 micron level are needed. Additionally, coverage of large solid angle requires chambers with the smallest possible dead space in the perimeter of the device. Ordinary proportional chambers usually feature massive frames in order to withstand the significant anode wire tension. The chambers under development in this project would provide all necessary functions required by a Muon System, i.e.

- Precision coordinate for the momentum determination (<70 microns)

- The transverse coordinate with coarser resolution (1mm or coarser, as required by the particular application)
- Bunch crossing timing (3 ns)
- Primitives for Level 1 trigger

In addition they will be constructed using modern, lightweight composite materials resulting in high precision construction necessary for achieving resolutions better than 100 microns while minimizing dead area in their perimeter.

Approach: The basis of our design is a low mass flat panel made of a parer (nomex) honeycomb core and copper-clad, 0.5 mm fiber epoxy facings forming the cathodes of the proportional chambers. These panels are approximately 2 cm thick and weigh about 1Kg per square meter. Three such panels, for example, would form a two layer chamber. One face in each gap is lithographically segmented into readout strips, typically, on a 5 mm pitch. Interpolation of the charge induced on these strips provides the precision momentum measurement coordinate. These panels are enclosed by suitable frames which provide the necessary features to complete the chambers. These are the 2.5mm steps for the attachment of the anode wires, the printed circuit boards for the electrical connections, gas manifolds, a gas seal, bolt holes, etc. These frames are quite narrow (less than 5 cm for example) because the panels are stiff enough to withstand the wire tension of about 60 Kg/m. Even though the size of the frames has thus been dramatically reduced, they would still dominate both the weight of the detectors as well as their cost if ordinary materials such as fiberglass epoxy composites requiring extensive precision machining are used. Multiple Coulomb scattering is also the dominant factor limiting the momentum

resolution of such detectors. In this project we have been investigating alternate materials and fabrication methods for the construction of proportional chambers. The use of lightweight polymer concrete, for example, has been a leading candidate in our investigation. Casting of the panels with such materials would drastically reduce the additional machining required, significantly reducing the fabrication cost. The initial cost of the casting forms, however, makes this techniques suitable for very large experiments where such a cost would represent a relatively small fraction of the total cost. Other materials such as rohacell foams are also being investigated for smaller experiments.

Technical Progress and Results: Industrial sources were investigated for the identification of appropriate casting materials that would form the panel closeouts. The desired properties of such materials were specified as follows:

- Low density (<0.5 gr/cc)
- Dimensional stability
- Low casting shrinkage
- Low creep
- Low thermal expansion coefficient
- Low viscosity
- High Young's modulus
- Dielectric constant less than 4
- High dielectric strength
- Good adhesion to other materials used in the panels, Al, Cu, G10 etc.

Several samples were fabricated in industry standard sizes appropriate for testing material properties. Most of these samples contained hollow glass microspheres in an epoxy matrix. The true particle density of these spheres is 0.25 gr/cc while their bulk density is 0.16 gr/cc. The samples were tested

at BNL. The most promising material identified has the following properties:

Composition, weight%	
Resin	53
Hardener	10
Glass spheres	37
Flexure strength, psi	1225
Flexual modulus of elasticity, psi	139,500
Compressive strength, psi	3,925
Young's modulus, psi	142,700
Density, gr/cc	0.55

In addition closed-cell, low density, high stiffness core materials such as rohacell made by Rohm Tech, Inc have been investigated and could be used also as more economical for smaller experiments. A prototype that could use either one of these techniques has been designed and is currently under construction. It is expected to be finished early in 1995. Cosmic ray tests followed by particle beam tests will be used to study the performance of this prototype.

FOLLOW-ON FUNDING:

Proportional chambers of this design have been accepted as the baseline technology covering the high pseudorapidity, eta, region of the Muon System of the ATLAS experiment at the CERN Large Hadron Collider. BNL participates in this experiment and, pending approval of the whole project, DOE funding for further development of these chambers would be a realistic possibility.

LDRD FUNDING:

FY 1994	\$ 87,527
FY 1995 (est.)	86,000

Theory of Self-Organized Criticality

Per Bak

94-34

PROJECT DESCRIPTION:

Self-organized criticality refers to the tendency of large dynamical systems to evolve to a critical state, with no characteristic length- or time-scale. The concept was conceived within the Condensed Matter Theory Group at BNL and has been applied to a great variety of natural phenomena, including geophysics, astrophysics, biology, and economics. Several mathematical models of self-organized criticality have been constructed at BNL and elsewhere, but, although significant progress has been done, our analytical understanding of those models is less than satisfactory. The objective of this project is to initiate a theoretical study of the underlying mechanisms for criticality. In particular, we plan to study models of interactive ecologies, and models of turbulent phenomena.

TECHNICAL PROGRESS AND RESULTS - Fiscal Year 1994:

Purpose: The purpose of the proposed research is to help in developing theories of models exhibiting self-organized criticality in order to enhance our understanding of complex dynamical systems. The funding supports Dr. Peter Thomas, who has been hired as a research associate for a period of two years.

Approach: The arrival of Peter Thomas was delayed, so that the present report deals with the progress made since his arrival.

(a) Numerical studies were performed on a model of biological evolution. The study

followed a discovery of Per Bak, in a collaboration with Kim Sneppen of the Bohr Institute, Denmark, of a simple mathematical

model of "punctuated equilibria". The concept of punctuated equilibria has been coined by Stephen J. Gould in order to describe that evolution is intermittent rather than gradual, with intermittent bursts of high activity. In the original model, the fitnesses of species were represented by random numbers. Peter Thomas studied a model where fitness is defined as a specific function of the genetic code. The model does not exhibit the expected behavior, but locks into some spurious oscillating states. We are re-evaluating how to proceed concerning the evolution models. However, we do not have a lack of models and problems related to other self-organized critical phenomena, but no firm decisions have been made yet.

(b) The phenomenon of "contact line depinning" has been studied, in a collaboration between Peter Thomas and Maya Paczuski. Contact line depinning is the phenomenon that the line separating a droplet on a surface is displayed intermittently as the droplet grows. The idea is to apply concepts derived from a more general study of surface depinning to this specific problem. This part of the project has just been initiated.

PAPERS/JOURNALS/PUBLICATIONS:

Peter Thomas has completed two manuscripts: "Exact results on a lattice model of a binary reactant mixture" and "Exchange effects in the Wigner Solid", on subjects indirectly related to the topic of this project.

LDRD FUNDING:

FY 1994	\$ 61,488
FY 1995(est.)	63,000

Development of the PCR-SSCP Technique for the Detection, at the Single Cell Level, of Specific Genetic Changes

K. Rithidech

94-36

PROJECT DESCRIPTION:

It has been well recognized that neoplastic transformation is initiated by gene-specific damage to single cells in the target tissue. However, accurate measurements of such damage in single cells cannot, at present, be made. Consequently, the risk to a particular cell of an untoward outcome resulting from injury caused by physical or chemical agents cannot be satisfactorily estimated. Thus, it is crucial that methods which allow direct identification of alterations at specific sites of the genome in each exposed cell, particularly those potentially associated with neoplastic transformation or other detrimental outcomes, be developed. In recent years, the polymerase chain reaction (PCR), in combination with other molecular techniques, has played a significant role in the molecular characterization of gene damage resulting from exposure to genotoxic agents. However, relevant studies have been generally carried out in heterogeneously pooled samples. Thus, it is necessary to establish PCR-based techniques for use in the detection of genetic damage in individual cells following exposure to toxic agents.

TECHNICAL PROGRESS AND RESULTS- Fiscal Year 1994:

Purpose: We propose to investigate the feasibility of using the polymerase chain reaction-single stranded conformational

polymorphism (PCR-SSCP) technique for the detection, following irradiation, of specific genetic changes in single cells. Although, the PCR-SSCP method has been successfully used to identify gene-harboring mutations in a pooled sample, its use in single cells has not as yet been reported. The PCR-SSCP technique is highly promising as an approach to identifying small numbers of cells harboring mutated genes. Also, it has the potential for use as a rapid molecular screening method for the early detection of cancer cells and thus for observing clonal expansion and progression. Such information has important applications for diagnostic, prognostic, and possibly therapeutic purposes, e.g., for the detection of drug resistant cells. More importantly, this study, if successful, will open a vital approach to the analysis of genetic damage at potentially critical sites for untoward outcomes following exposure to genotoxic agents at the single cell level. Using this approach, an evaluation of subpopulations of sensitive or resistant cells is also feasible.

The detection of genetic damage using the PCR-SSCP technique depends on the fact that induced base alterations in the single strands of DNA lead to changes in their three dimensional conformation. These changes produce electrophoretic mobility shifts which can be observed using a non-denaturing electrophoretic technique. This technique can detect many types of base damage such as point mutations, small deletion or inversion, and gene loss. It is rapid, extremely sensitive, specific, and many samples can be analyzed simultaneously. Furthermore, the entire gene can be amplified and then digested with restriction enzymes for further SSCP analysis. With the availability of appropriate and specific DNA probes, the PCR-SSCP method could replace the time consuming and non-specific assays currently used for the detection

of genetic damage such as cytogenetic methods and the microgel electrophoresis technique.

Approach: In the first year of the project, we shall focus on the establishment of the PCR-SSCP methodology using pooled samples. These samples are radiation-induced myeloid leukemic (ML) cells that were collected from mice with ML resulting from exposure to monoenergetic fast neutrons, γ rays, or X rays over the years during our ongoing studies on radiation leukemogenesis. These ML cells are cryopreserved and suitable for later DNA analyses. They are also perpetuated by transplantation into syngeneic mice. We shall apply the PCR-SSCP for study N-ras mutations in DNA isolated from these ML cells. Subsequently, we shall demonstrate that the PCR-SSCP analysis can be used to detect N-ras mutations in DNA isolated from cells of mice exposed to different doses of X rays. The N-ras gene is selected for our initial study because it is known to play an important role in both murine and human oncogenesis. Furthermore, this gene has been extensively studied; therefore, its DNA sequence, mutational spectra, and appropriated DNA primers for PCR amplification have been well characterized.

Following the establishment of the PCR-SSCP method for the detection of N-ras mutations, we shall illustrate further that a selected region of another gene, i.e. a 480 base-pair long, portion of Exon 9 of the mouse hypo-xanthine-guanine phosphoribosyl transferase (HPRT) gene, can be amplified in both normal and X-irradiated cells. Thereafter, we shall use the SSCP method for the detection of damage at this specific site in these PCR products. The HPRT gene is included in this project because it is the only locus that can be used for studying in vivo mutagenesis, both in human beings and

experimental animals. Furthermore, its mutations has been known to be associated with the human Lesch-Nyhan syndrome.

In the second year of this project, we shall apply the PCR-SSCP technique for the detection of gene changes following exposure to X rays at a single cell level. To do this, it is imperative that we first optimize the PCR conditions for amplifying selected genes, i.e. N-ras and HPRT genes, in single cells. We shall begin with cells from non-exposed mice. A serial dilution or micromanipulation will be used to isolate single cells from the mouse bone marrow. Careful experimental strategies will be implemented to circumvent potential shortcomings of the use of PCR in single cells. After the development of a single cell PCR has been achieved, we shall continue on experiments involving the use of SSCP analysis for the detection of N-ras or HPRT mutations in DNA isolated from mice exposed to different doses of X rays on a cell-by-cell basis.

Technical Progress and Results: The PCR-SSCP technique is tremendously sensitive for the detection of base changes. However, it has some technical limitations. These include conditions for PCR amplification (e.g. effects of primers and annealing temperature) and for SSCP analysis (e.g. effects of polyacrylamide concentration in the gel, power, temperature, and gel running time). In spite of these technical difficulties, we have made steady progress in establishing the PCR-SSCP technique for the detection of mutations at specific sites of the genome, based on pooled samples.

(a) We used this technique successfully as a rapid screening method for N-ras gene mutations in radiation-induced ML cells. We focused our analyses to: i) a 128 bp long, portion of Exon I (codons 2-37); i) and 103

bp long, portion of exon II (codons 48-82). Twelve cases of radiation-induced ML were included in this study (4 neutron- and 8 photon-induced ML). No mobility shifts were detected with Exon I DNA; however, mobility shifts of Exon II DNA bands were observed in all ML samples (Fig. 1). These results indicate the involvement of mutations in Exon II, but not in Exon I, of the N-ras gene in radiation leukemogenesis. Moreover, the mobility of single strand DNA isolated from neutron-induced ML was distinct from that of photon-induced ML. This finding suggests that mutational spectra of the N-ras gene differ in ML induced by different types of radiation. Although mutation types can be determined by means of a direct sequencing method, such studies are not included in the scope of this project. However, PCR products obtained from these experiments are stored for later DNA sequencing.

(b) We were able to use the PCR-SSCP method for the detection of N-ras changes in DNA isolated from mice exposed (whole body) to different doses of X rays. In these experiments, we focused our analyses to only the 103 bp long, codons 48-82, of Exon II of the N-ras gene. It was evident that a fragment of, approximately, 103 bp was amplified from DNA isolated from mice exposed to 0, 0.5, 1.0, 4.0, 10.0 Gy of X rays, respectively. It is possible, however, that these DNA fragments may represent products from: i) only wild-type cells, or ii) a mixed population of wild-type and damage cells, or iii) damage cells containing a few basepairs change which do not interfere with the amplification process. Results from the SSCP analysis of these PCR products excluded possibility #i because mobility shifts were observed in all DNA from mice exposed to X rays (as low as to 0.5 Gy X rays). Although types of mutations are not studied in these samples, the results provided very important information regarding the

"target" size of the genome affected by X-irradiation.

(c) We successfully demonstrated that a 480 bp long, a portion of Exon 9 of the mouse HPRT gene can be amplified from DNA of mice exposed *in vivo* to different doses of X rays. To increase sensitivity of the SSCP analysis, the 480 bp PCR products were digested with the restriction enzyme *HindIII* resulting in 2 fragments of 172 and 308 bp. We used the SSCP analysis to determine if there was any base change in the 172 bp fragment. Again, results clearly showed the sensitivity of the SSCP analysis because mobility shifts were observed in all DNA isolated from exposed mice (Fig. 2). Currently, the SSCP analysis is being conducted on the 308 bp fragments.

PAPERS/JOURNALS/PUBLICATIONS:

An article entitled, "Detection of N-ras gene mutations in radiation-induced murine myeloid leukemia by the PCR-Single strand based technique" was presented at the 25th Annual Meeting of the *ENVIRONMENTAL MUTAGEN SOCIETY*, May 7-12, 1994, Portland OR. This article was also published in *Environmental and Molecular Mutagenesis*, Vol. 23, Suppl. 23:57, 1994.

FOLLOW-ON FUNDING:

We plan to submit a grant proposal entitled, "Measurement of Genetic Damage in Single Mouse Bone Marrow Cells Using PCR Based Techniques" to NASA or EPA.

LDRD FUNDING:

FY 1994	\$ 85,114
FY 1995 est.	88,300

Note: This project involves vertebrate animals.

Fig. 1. Detection of mutations in 103bp long of Exon II of mouse N-ras by PCR-SSCP analysis. Lane 1 is PCR products of neutron-induced ML cells. Lanes 2-3 are PCR products of γ -ray-induced ML cells, lanes 4-7 were PCR products of x-ray-induced ML cells, and lane 8 is the PCR product of control mouse DNA cells.

Fig. 2. PCR-SSCP analysis of mutations in the 172bp long of Exon 9 mouse HPRT gene. DNA samples were isolated from mice exposed to different doses of x rays, i.e., 0 Gy (lanes 1-2), 0.5 Gy (lanes 3-4), 1.0 Gy (lanes 5-6), 4.0 Gy (lanes 7-8). Samples were loaded with denaturation (lanes 2,4,6, and 8) and without denaturation (lanes 1,3,5, and 7).

Feasibility of SPECT in Imaging of F-18 FDG Accumulation in Tumors

Gene-Jack Wang

94-37

PROJECT DESCRIPTION:

To evaluate the feasibility of SPECT in imaging of ^{18}F FDG accumulation in tumors, we compare the sensitivity and specificity of PET and SPECT on ^{18}F FDG imaging of breast tumors. Patients who are suspected of having breast cancer following mammography will be recruited for this study. The patients will have a PET scan following the injection of ^{18}F FDG. Images of chest and axillary region will be obtained using a SPECT system with high energy collimators following the PET scan.

TECHNICAL PROGRESS AND RESULTS - Fiscal Year 1994:

This project has been delayed due to lack of an adequate SPECT scanner at BNL. We have, therefore, decided to conduct this study at Stony Brook University Hospital where such a scanner is available. We are planning to resume the project when the high energy collimators for the SPECT system in the University Hospital is installed.

LDRD FUNDING:

FY 1994	\$ 15,806
FY 1995 est.	100,000

Note: This project involves the use of human subjects

Feasibility Studies for Establishing a Proton Facility for Cancer Therapy

A. D. Chanana

94-42

PROJECT DESCRIPTION:

The purpose of this work is to undertake a detailed evaluation of the scientific and technical issues relevant to the establishment of a proton therapy facility at BNL using AGS Linac. The issues to be discussed in detail include: a) compatibility with Booster-AGS-RHIC and BLIP operations. b) Beam transport and beam scanning options. c) The gantry design.

TECHNICAL PROGRESS AND RESULTS - Fiscal Year 1993:

Purpose: Despite advances in cancer therapy with photons (high energy medical accelerators, 3-D treatment planning, and conformal treatment with multileaf collimators), local tumor control fails each year in ~260,000 patients in the USA and radiation-induced morbidity due to unavoidable irradiation of the normal tissue also remains a formidable problem. The superior dose distribution achievable with protons permits much tighter irradiation of the target volume with greater sparing of the surrounding healthy tissue primarily because with protons, and for each beam path, there is no dose beyond the target. Furthermore, improvements in tumor localization, when combined with the progress in coregistration of imaging modalities such as PET, SPECT, CT, and MRI allow full exploitation of the superior dose distribution characteristics of protons.

Approach: A major leadership effort will be required to properly plan and coordinate the various issues and activities inherent in

establishing a proton therapy facility at BNL. Initially, the P.I. of this LDRD program, working with BNL medical physicists, accelerator physicists, health physicists and the staff of the Plant Engineering Division, shall develop a Pre-conceptual Design Report for a Proton Therapy Facility at BNL. During the development of this report, advice and input will also be sought from the staffs of the existing proton therapy facilities in U.S.A. and abroad.

Technical Progress and Results: The major technical and scientific issues including those concerned with the beam transport, beam scanning and gantry design have been resolved. The short- and long-term research and development efforts required for the proposed proton therapy facility have been identified. The scope of the conventional engineering effort for the proposed facility has been delineated. The results from the current studies fully support the following conclusion from the preliminary study: 'The Laboratory is well positioned to develop a state-of-the-art proton therapy facility'.

The Pre-conceptual Design Report for the proposed proton therapy facility is under preparation and is expected to be ready for submission to the Laboratory Director during this calendar year (1994).

PAPERS/JOURNALS/PUBLICATIONS:

"Proposal for the use of the AGS Linac for Proton Therapy Facility", by D. Raparia, J.G. Alessi, A.D. Chanana, F.A. Dilmanian, Y.Y. Lee, and L. Wielopolski. Brookhaven National Laboratory, Upton, NY. Presented at the 17th International Linac Conference (LINAC 94), Tsukuba, Japan, August 21-26, 1994.

LDRD FUNDING:

FY 1994

\$ 176,895

Visible Free-Electron Laser Experiment

I. Ben-Zvi

94-43

PROJECT DESCRIPTION:

A Free-Electron Laser (FEL) oscillator is being studied at the short wavelength limit of the electron-beam-brightness. The wavelength is made short through the use of a very short-period undulator (micro-undulator). The 50 to 70 MeV electron beam of the BNL Accelerator Test Facility is being used in beam-line 3 of the ATF. Two types of undulators are available for the experiment: a pulsed electromagnet undulator made by MIT and a superferric undulator built at BNL. Both have a period of 8.8 mm. A successful demonstration of this short wavelength FEL oscillator will be an important step towards the realization of high power, tunable very short wavelength radiation sources in the VUV and X-rays. These sources may have important R&D applications in photochemistry, atomic and surface physics, biology and other sciences.

TECHNICAL PROGRESS AND RESULTS - Fiscal Year 1994

Purpose: In recent years, the emergence of research applications requiring intense, coherent, high brightness radiation sources beyond existing state of the art, has driven

a wave of accelerator research and development. The NSLS has developed a proposal for the construction of a Deep Ultra-Violet FEL. An advisory panel chaired by Andrew Sessler of LBL recommended, among other things, the demonstration of the Visible FEL Oscillator Experiment. The motivation is the operation of a FEL near the emittance limit of the accelerator and, possibly, extension to the near UV provided the beam brightness is as calculated.

Approach: The hardware of the Visible Free-Electron Laser experiment includes two 60 cm long microundulator with a period of 0.88 cm. One is a BNL built superconducting device and the other is a MIT built pulsed device. The superconducting undulator technology has been developed at the National Synchrotron Light Source at BNL under a previous LDRD (91-22) project. We use a ferromagnetic yoke machined out of a solid block of low carbon steel. A superconducting NbTi coil is wound continuously along the yoke, with the winding direction alternating every half period. The magnetic field of this undulator is very uniform even for operation above saturation. The experiment is being installed on beam line number 3 of the BNL Accelerator Test Facility (see figure). The FEL output will be at a wavelength of 500 nm at an electron beam energy of 50 MeV. The planned upgrade of the ATF to 70 MeV will make it possible to extend the operation of this FEL to the UV, about 250 nm. The FEL interaction

and resonator design were studied in detail. An output power of 10 MW peak and a gain of about 25% are expected at the ATF beam parameters but with only 50 A peak current and a rms normalized emittance of 7p mm mrad.

Technical Progress and Results: The beam line of the experiment has been assembled and surveyed, including support stands, beam line magnets (dipoles, quadrupoles, steerers), vacuum hardware, electron beam diagnostics, beam dump and optical diagnostics. The vacuum system has been built and operated.

The MIT pulsed microundulator has been measured, installed and surveyed. A number of ATF accelerator runs were done and the beam transport was studied. Spontaneous emission has been measured out of the microundulator. The spectrum was at the calculated wavelength but broader than expected. The reason for the broad spectrum has been found to be instability in the ATF power klystron. During the last maintenance period this klystron has been replaced and a feed-forward control system is being developed to control the power stability of the klystron.

In addition to the klystron work, a few other improvements have been made to the ATF systems that have a direct influence on the FEL performance. The energy has been increased to about 70 MeV (previously it was only 50). This will enable us to try for FEL lasing at shorter wavelength yet, down to 250 nm. To put this in perspective, the current world record for the shortest linac-based FEL is about 350 nm (Los Alamos) and the shortest wavelength for any FEL is 240 nm (Novosibirsk). The low energy injection

system of the ATF has been upgraded to include an emittance correction system. The improved emittance will be necessary to get the extra gain needed at the intended UV wavelength, where the emittance is more critical and mirrors have larger losses.

The Nd:YAG laser that drives the ATF photocathode rf gun has been upgraded as well. The new laser is much more stable in phase and amplitude, requires less attention to operate and has a unique pulse train generation and shaping for controlling the pulse train for the FEL operation.

Following the resumed operation of the ATF with all these improvements, we will begin the electron beam measurements. Emittance, peak current, energy and proper beam optics will be measured and checked against the specified levels needed for the FEL. The beam diagnostic devices needed for this work are for the most part in place and work is in progress on the rest.

Currently work is being carried out on precision alignment of the FEL. A green HeNe laser alignment system has been installed. With this system we can align with a very high precision the cavity mirrors, the electron beam line and the undulator magnetic axis to coincide. As an extension of this method we will try an interferometric system to bring the cavity mirrors into precise alignment.

We are preparing a laser based cavity length measurement. For this purpose we will inject the green (one doubled) beam of the Nd:YAG drive laser into the cavity.

Using the pulse shaping available in this laser, we will be able to measure the ringing of the cavity and bring it to resonate with the Nd:YAG repetition frequency. With these initial alignment methods, we should be able to achieve lasing quickly, once the electron beam has been characterized.

LDRD FUNDING:

FY 1994
FY 1995 est.

\$ 70,000
100,000

Figure 1. The FEL oscillator

Brookhaven National Laboratory

Future

***O*n the previous page:**

An artist's rendition of the BNL Imaging and Neuroscience Center, which will house the new 4 tesla magnet Magnetic Resonance Imaging (MRI) facility. When completed in 1995, the Center will contain three imaging facilities for Position Emission Tomography (PET), Single Photon Emission Computed Tomography (SPECT) as well as MRI. The success of the MRI program is due in great part to the LDRD project reported herein, "Relaxographic MRI and Functional MRI", #93-35.

Summary

In FY 1994, the BNL LDRD Program funded 27 projects, 15 of which were new starts, at a total cost of \$2,322,630. Following is a table, which lists all of the FY 1994 funded projects and gives a history of funding for each by year.

Several of these projects have already experienced varying degrees of success, as indicated in the preceding individual Project Program Summaries. A total of 34 informal publications (abstracts, presentations, BNL reports and workshop papers) were reported and an additional 39 formal (full length) papers were either published, in press or being prepared for publication. The investigators on one project (#93-33) have filed for a patent. On another project (#93-24) the investigator has received the Established Investigator Award from the National Alliance for Research on Schizophrenia and Depression (NARSAD), along with a \$68,512 grant.

Fourteen of the projects reported that proposals, grants and pre-conceptual designs had either been funded or were submitted for funding. The complete summary of follow-on activities is as follows:

Follow-on Activity of LDRD Projects	
	Number of Projects
Informal Publications	34
Formal Papers	
Follow-on Funding	4
Proposal to DOE	9
Grants/Proposals to other Agencies/Organizations	6
Facility Funded	1
Pre-conceptual design submitted	1
CRADA Application Proposed	1

In addition, numerous post doctoral candidates and guest scientists were supported or collaboratively involved in these project.

In conclusion, a significant measure of success is already attributable to the FY 1994 LDRD Program in the short period of time involved. The Laboratory has experienced a significant scientific gain by these achievements.

**LABORATORY DIRECTED RESEARCH AND DEVELOPMENT
FISCAL YEAR 1994
SUMMARY OF PROJECTS**

PROJ. NUMBER	PROJECT TITLE	PRINCIPAL INVESTIGATOR	FY 91	FY 92	FY 93	FY 94	FY 95	TOTAL
91-21	Effect of a Bacterial Spore Protein on Mutagenesis	J. Setlow	\$ 87,461	\$159,594	\$161,915	\$ 30,155*	0	\$439,125
92-15	Studies on the Cellular Toxicity of Cocaine & Cocaethylene	N. Volkow	0	31,837	44,028	13,623	0	89,488
93-10	Calcification in Marine Alga: A Molecular Approach to a Major Pathway of Global Carbon Cycling	J. LaRoche	0	0	58,370	60,515	0	118,885
93-11	Structure-Sensitive Properties of Advanced Permanent Magnet Materials: Experiment and Theory	D. Welch	0	0	99,187	105,710	0	204,897
93-20	A High Flux Neutron Source	J. Powell	0	0	38,895	44,306	0	83,201
93-22	Genetics of Drug Addiction	C. Ashby	0	0	68,023	87,391	0	155,414
93-24	Development of Microdialysis Technology	S. Dewey	0	0	50,150	61,526	0	111,676
93-25	Investigation of the Utility of Maximum-Entropy Methods for the Analysis of Powder Diffraction Data	D. Cox	0	0	24,431	73,342	40,000	137,773
93-30	Accelerator Technology	A. Ruggiero	0	0	153,210	249,900	0	403,110
93-32	Analysis of Structures and Interactions of Nucleic Acids and Proteins by Small Angle X-ray Diffraction	M. Capel	0	0	42,276	97,690	48,000	187,966
93-33	Enhancement of Microplanar Beam Radiation Therapy (MRT) of the Intracerebral Rat 9L Gliocarcinoma by Preinfusion with a Diiodofluorescein-Histone Conjugate	D. Slatkin	0	0	42,638	96,966	0	139,604
93-35	Relaxographic MRI and Functional MRI	J. Fowler	0	0	8,675	93,408	85,000	187,083
94-03	Very Low Temperature Infra-red Laser Absorption as a Potential Analytical Tool	G. Hall	0	0	0	57,142	59,600	116,742
94-05	State-resolved Measurements of H ₂ Photodesorption: Development of Laser Probes of H ₂ for In-situ Accelerator Measurements	M. White	0	0	0	\$ 68,871	77,300	146,171
94-06	Siberian Snake Prototype Development for RHIC	M. Harrison	0	0	0	132,139	134,000	266,139

[illegible]