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Laboratory Directed Research & Development Program

Annual Report to the Department of Energy

December 1992

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Associate Director for Administration

BROOKHAVEN NATIONAL LABORATORY
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LDRD COMMITTEE MEMBERS

Martin Blume	**Deputy Director, Chairperson**
John D. Axe	**Associate Director for Basic Energy Sciences**
Seymour Baron	**Associate Director for Applied Programs**
Henry C. Grahn	**Associate Director for Administration**
Mark Sakitt	**Assistant Director for Planning & Policy**
Melvin Schwartz	**Associate Director for High Energy & Nuclear Physics**
Richard B. Setlow	**Associate Director for Life Sciences**

INTRODUCTION

Brookhaven National Laboratory has three primary missions. The first is to conceive, design, build and operate in a safe and environmentally sound manner, complex research facilities for the benefit of the entire scientific community. These facilities, such as particle accelerators and colliders, nuclear reactors and synchrotron storage rings are used for fundamental scientific studies and both basic and applied research in energy-related, physical, life and environmental sciences.

The second mission is to carry out basic science research efforts in long-term high-risk programs which have potential long-term payoffs. Many of these programs employ the unique facilities mentioned above; others take advantage of the special expertise and ancillary support services and facilities at the Laboratory. The ease of engaging in collaborative efforts with outside users from universities, industries and other government laboratories greatly enhances the effectiveness of the programs and encourages wide based use of the special facilities present at Brookhaven.

The third mission is to contribute to the technology base of the nation. The Laboratory is engaged in the development of new technologies and facilities. The process of transferring this new knowledge to the commercial sector helps advance and broaden the nation's present basic and applied research capabilities. Brookhaven is involved in the education of scientists and engineers through a wide variety of cooperative research programs and has other extensive educational programs covering a broad spectrum, reaching elementary school through university students and faculty.

As a national resource, Brookhaven makes available, when feasible, its unique facilities and expertise to state and federal agencies and to the private sector.

Today, 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 these new ideas and technologies, promotes the early exploration and exploitation of creative and innovative concepts, and which develops new "fundable" R&D projects and programs. At Brookhaven National Laboratory (BNL), one such method is through its Laboratory Directed Research and Development (LDRD) 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 Department of Energy (DOE) and the Brookhaven National Laboratory.

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 formally established under the guidelines set forth in DOE Order 5000.1 in May 1984. From inception through September 1992, a period spanning eight fiscal years, the Laboratory has authorized $17.7 million in Exploratory R&D, consisting of 121 separate projects.

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.

PROGRAM STRUCTURE: The LDRD Program does not have any formalized substructure in that there are not defined a priori any separate or distinct categories of projects. All projects and their proposals, large and small, regardless of institutional purpose or potential impact, are treated similarly in the Program's administrative procedures.

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 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 Associate Director for Administration, who 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 six members are: the Associate Director for High Energy and Nuclear Physics; the Associate Director for Applied Programs; the Associate Director for Life Sciences; the Associate Director for Basic Energy Sciences; the Associate Director for Administration; and the Assistant Director for Planning & Policy.

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 Committee; the overall financial health of the Laboratory; and a number of budgetary tradeoffs between LDRD and other overhead expenses.

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 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.

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 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 LDRD Committee who transmits a copy of all proposals received to the LDRD 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 LDRD 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 5000.4 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 39 percent for new starts. From inception of the program through September 1992, 307 proposals were considered and 121 were approved.

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 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 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.

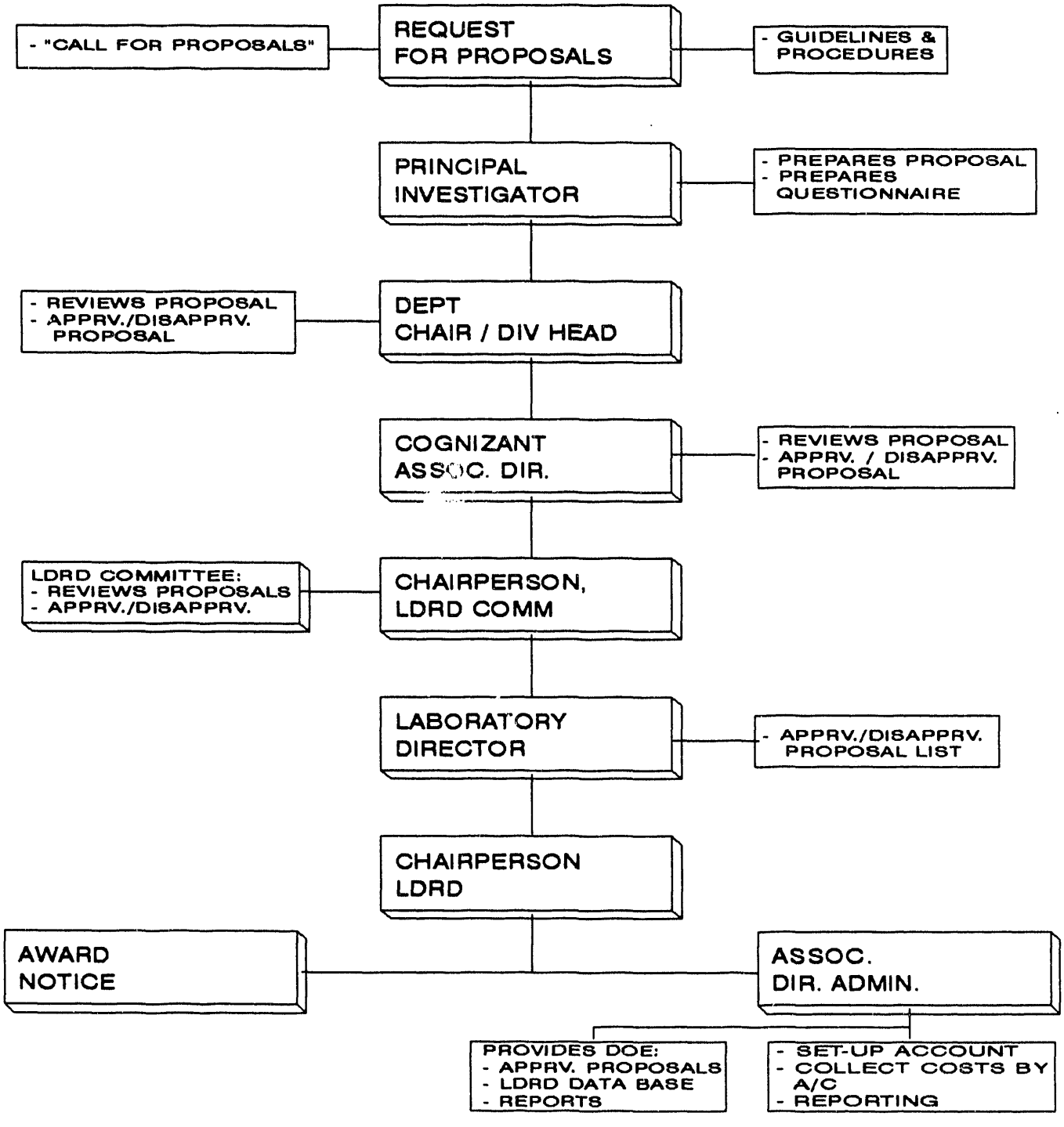
The manager ensures that the work is completed in a timely manner and that annual status reports are submitted to the Deputy Director. In addition, LDRD Program activity is reported to the DOE Area Office Manager, including copies of all funded proposals, a LDRD Program data base, and a project funding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

LABORATORY DIRECTED RESEARCH AND DEVELOPMENT PROGRAM PROCESS



LABORATORY DIRECTED RESEARCH AND DEVELOPMENT PROGRAM

($000)

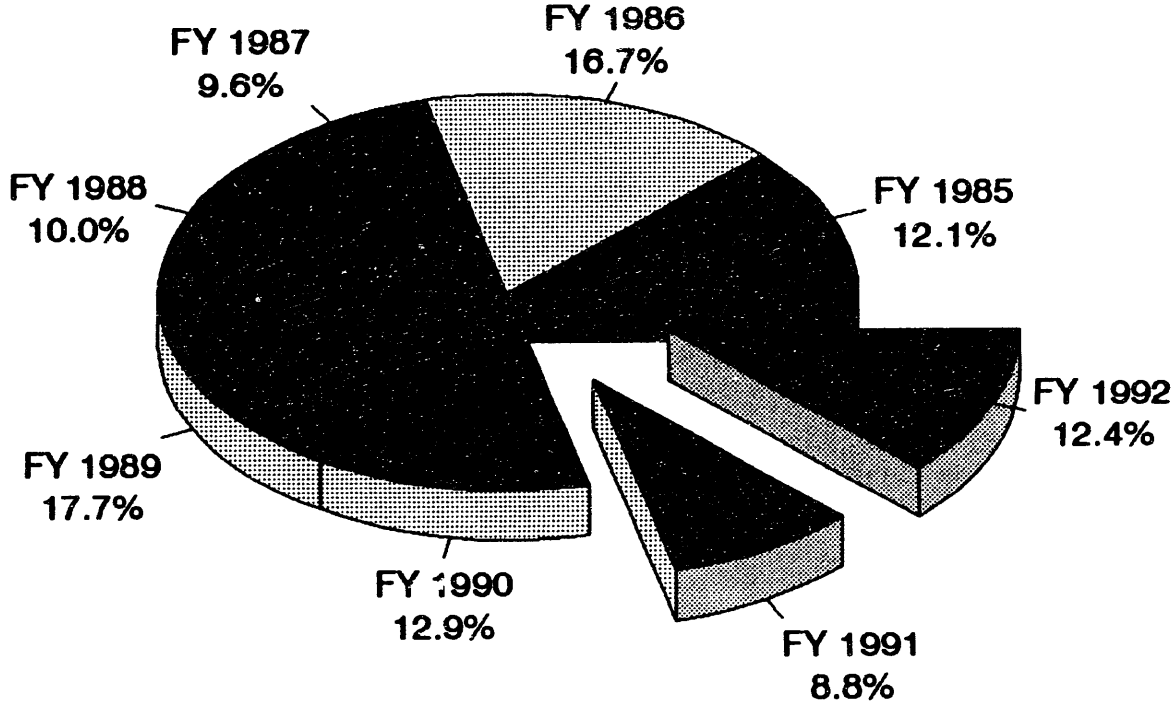
	FY 1985	FY 1986	FY 1987	FY 1988	FY 1989	FY 1990	FY 1991	FY 1992
DOE	$153,000	$156,500	$161,700	$176,700	$193,600	$203,800	$220,900	$234,300
WFO	$40,400	$45,100	$45,600	$45,900	$46,700	$45,200	$50,300	$47,200
TOTAL OPERATING	$193,100	$201,600	$207,300	$222,600	$240,300	$249,000	$271,200	$281,500
LDRD FUNDING	$1,819	$2,515	$1,443	$1,510	$2,666	$1,941	$1,321	$1,865
% OF OPERATING FUNDS	.9 %	1.2 %	.7 %	.7 %	1.1 %	.8 %	.5 %	.7 %

LABORATORY DIRECTED RESEARCH AND DEVELOPMENT FUNDING BY FISCAL YEAR

FISCAL YEAR	FUNDING ($000) DOE CEILING	FUNDING ($000) BNL AUTHORIZED	FUNDING ($000) COSTED	PROPOSALS NO. CONSIDERED	PROPOSALS NO. OF NEW STARTS
1985	$4,000	$1,842	$1,819	39	13
1986	$4,500	$2,552	$2,515	22	15
1987	$4,000	$1,451	$1,443	29	8
1988	$4,000	$1,545	$1,510	46	14
1989	$4,000	$2,676	$2,666	42	21
1990	$4,000	$2,008	$1,941	47	9
1991	$2,000	$1,353	$1,321	23	14
1992	$2,500	$1,892	$1,865	30	14
TOTALS		$15,319	$15,080	278	108

LABORATORY DIRECTED RESEARCH AND DEVELOPMENT PROGRAM

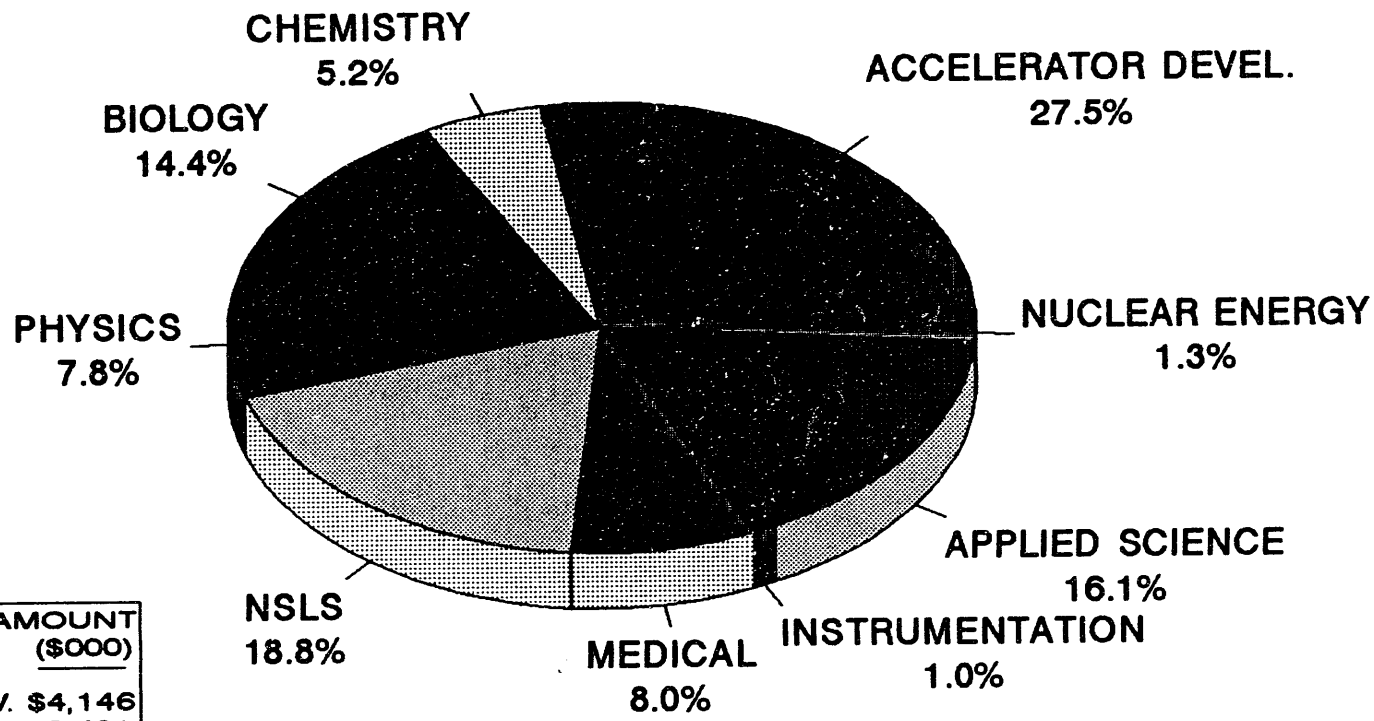
APPROVED PROJECTS BY FISCAL YEAR FROM INCEPTION:



FISCAL YEAR	COST ($000)
1985	$1,819
1986	2,515
1987	1,443
1988	1,510
1989	2,666
1990	1,941
1991	1,321
1992	1,865
TOTAL	$15,080

LABORATORY DIRECTED RESEARCH AND DEVELOPMENT PROGRAM

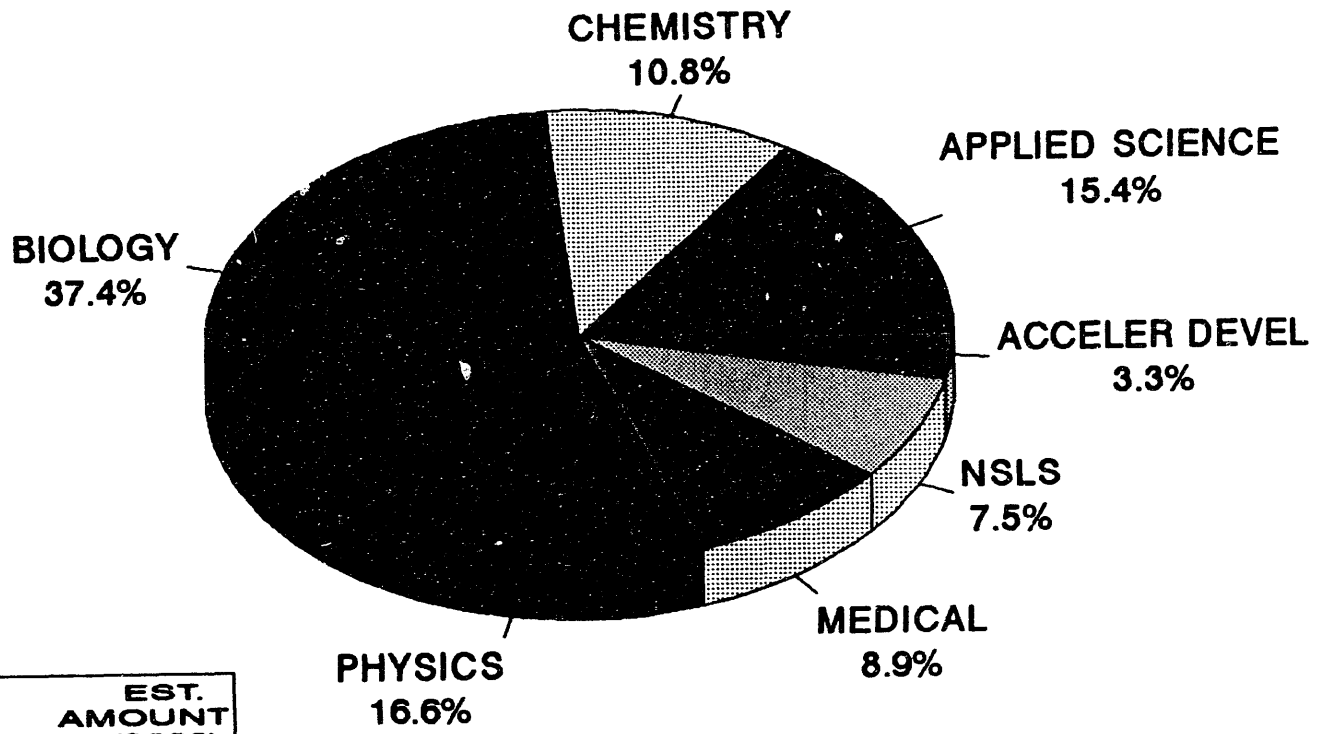
FUNDED PROJECTS BY DEPT./DIV. FROM INCEPTION:



DEPT./DIV.	AMOUNT ($000)
ACCELERATOR DEV.	$4,146
APPLIED SCIENCE	2,431
BIOLOGY	2,171
CHEMISTRY	782
INSTRUMENTATION	145
MEDICAL	1,205
NSLS	2,838
NUCLEAR ENERGY	193
PHYSICS	1,169
TOTAL	$15,080

LABORATORY DIRECTED RESEARCH AND DEVELOPMENT FUNDED PROJECTS BY DEPARTMENT

FISCAL YEAR 1992:



DEPT./DIV.	EST. AMOUNT ($000)
ACCELER DEVEL	$ 62
APPLIED SCIENCE	288
BIOLOGY	697
CHEMISTRY	202
MEDICAL	166
NAT'L SYNCHROTRON LIGHT SOURCE	140
PHYSICS	310
TOTAL	$1,865

LABORATORY DIRECTED RESEARCH & DEVELOPMENT PROGRAM
FISCAL YEAR 1992

SUMMARY PROJECTS

PROJECT CONTROL NO.	PROJECT TITLE	FY90	FY91	FY92	FY93	FY94	TOTAL
90-41	Advances in Geoexploration	$ 88,427	$ 99,709	$ 60,118	$ 0	$ 0	$ 248,254
90-44	Transvenous Coronary Angiography with Synchrotron x-ray	41,070	29,043	1,718	0	0	71,831
90-45	Borehole Measurements of Global Warming	88	34,913	49,518	0	0	84,519
91-03	Molecular Ecology: Development of Field Methods for Microbial Growth Rate and Activity Measurements	0	49,229	50,602	0	0	99,831
91-04	A New Malaria Enzyme - A Potential Source for a New Diagnostic Test for Malaria and a Target for a New Antimalarial Drug	0	52,763	55,875	59,000	0	167,638
91-07	Basic Studies on Thoron and Thoron Precursors	0	37,941	51,991	0	0	89,932
91-08	Cloning of the cDNA for a Human Serine/Threonine Protein Kinase that is Activated Specifically by Double-Stranded DNA	0	85,318	89,466	0	0	174,784
91-18	Development of an Ultra-Fast Laser System for Accelerator Applications	0	79,657	99,993	0	0	179,650
91-19	Cluster Impact Fustion	0	29,696	49,766	0	0	79,462
91-21	Effect of a Bacterial Spore Protein on Mutagenesis	0	87,461	159,594	162,000	0	409,055

LABORATORY DIRECTED RESEARCH & DEVELOPMENT PROGRAM
FISCAL YEAR 1992

SUMMARY PROJECTS
(Continued)

PROJECT CONTROL NO.	PROJECT TITLE	FY90	FY91	FY92	FY93	FY94	TOTAL
91-23	Structure and Function of Adenovirus Penton Base Protein	$ 0	$ 64,623	$ 138,877	$ 65,000	$ 0	$ 268,500
92-04	High Resolution Fast X-ray Detector	0	0	57,542	0	0	57,542
92-06	Coherent Synchrotron Radiation Longitudinal Bunch Shape Monitor	0	0	40,000	0	0	40,000
92-07	High Gain Harmonic Generation Experiment	0	0	100,000	100,000	0	200,000
92-09	BNL Maglev Studies	0	0	59,548	40,000	0	99,548
92-13	Structural Investigations of Pt-Based Catalysts	0	0	68,334	73,000	0	141,334
92-15	Studies on the Cellular Toxicity of Cocaine and Cocaethylene	0	0	31,837	45,000	0	76,837
92-17	Human Melanocyte Transformation	0	0	81,463	91,000	0	172,463
92-19	Exploratory Applications of X-Ray Microscopy; Determination of the Higher Order Structure of Eukaryotic Chromosones	0	0	74,783	80,000	0	154,783

LABORATORY DIRECTED RESEARCH & DEVELOPMENT PROGRAM
FISCAL YEAR 1992

SUMMARY PROJECTS
(Continued)

PROJECT CONTROL NO.	PROJECT TITLE	FY90	FY91	FY92	FY93	FY94	TOTAL
92-20	Uranium Neutron Capture Therpy (UNCT)	$ 0	0	97,021	104,000	0	201,021
92-22	Tunneling Microscopy Studies of Manoscale Structures	0	0	84,860	87,000	0	171,860
92-27	Nuclear Techniques for Study of Biological Channels	0	0	68,398	70,000	0	138,398
92-28	RF Sources for Accelerator Physics (CAP)	0	0	98,792	100,000	0	198,792
92-29	Induction and Repair of Double-Strand Breaks in the DNA of Human Lymphocytes	0	0	132,861	0	0	132,861
92-30	An EBIS Source of High Charge State Ions up to Uranium	0	0	61,548	66,000	0	127,548
		$ 129,585	$ 650,353	$1,864,505	$1,142,000	$ 0	$3,786,443

PROJECT NUMBER: 90-41

PROJECT TITLE: Advances in Geoexploration

PRINCIPAL INVESTIGATOR: Manowitz, B.

LDRD FUNDING:

FISCAL YEAR		AMOUNT
1990		$ 88,427
1991		99,709
1992		60,118
1993	(authorized)	0
1994	(estimated)	0
TOTAL		$248,254

PROJECT DESCRIPTION:

Useful advances in geoexploration include the confirmation of the validity of simple biomarkers for hydrocarbon source identification and the development of simple and rapid techniques for the analysis of hetero and organometallic compounds in complex hydrocarbon mixtures, such as EXAFS, and the development of simple models for estimating potential hydrocarbon accumulations in frontier areas. Many problems in the evolution, migration, and alteration of petroleum can be elucidated by sulfur isotope studies of crude oils. Natural variations in sulfur isotope ratios are:
1) strongly dependent on the initial biological source input;
2) independent of thermal maturation except in the presence of reservoir sulfate;
3) are independent indicators of source rocks even where significant migration has taken place, and
4) in conjunction with other characterization parameters can be indicative of secondary reservoir biological activity and of water washing.

In a recent work, the hypothesis was developed that sulfur isotope evidence for biodegradation and water washing can be interpreted by determining the ratio of aliphatic to aromatic compounds in the same suite of oils. It is proposed to conduct such an analysis by a comparison of EXAFS data on model sulfur compounds and on the 27 oils previously analyzed for sulfur isotopes. This study will further demonstrate the usefulness of sulfur data as independent correlational parameters. The EXAFS sulfur studies will be extended to other suites of crude oils. In an ancillary effort, a simple model has been developed that estimates hydrocarbon accumulations in specific regions of the Delaware Basin and the Trans-Pecos Texas area. The generation and preservation predictions are compared to integrated production data for those Basins. The most uncertain variable-carbon, sequestering efficiency, is adjusted so that the hydrocarbons accumulated are in a range appropriate to the hydrocarbons produced and the assumptions for the adjustment are geophysically justified. It is proposed to extend such analysis to other regions where production data are available to see whether consistent assumptions to those made in the simple model presented are sufficiently valid that applications of the model can be made to selected frontier regions.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: A unique methodology for speciating sulfur compounds and other light element compounds in complex hydrocarbon mixtures is by X-Ray Absorption Near Edge Structures (XANES) spectroscopy. The experiment involves scanning through the K- or L-shell absorption edge of the element in question. The structure of the absorption edge, consisting of transitions to unoccupied molecular levels, can be compared to those of model compounds for identification. The relative position of the absorption edge can yield information regarding the oxidation state of the element. This portion is the XANES portion of the spectrum. The Extended X-Ray Absorption Fine Structure (EXAFS) region, extending from about 60 eV above the absorption edge, represents scattering from neighboring constituents and can be used to determine the coordination number and coordination distance of a specific element from its neighboring atoms

APPROACH: The best source of excitation energy for these experiments is an electron storage ring emitting synchrotron radiation (SR). The National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory is a 2.5-GeV storage ring and emits a continuous spectrum of x-rays to an energy of about 30 keV. Beam line X-19A is dedicated to XANES and EXAFS and is being adapted to the performance of this investigation.

In the X-19A beam line, x-rays are transported from the bending magnet source to the measuring apparatus through the system shown in Figure 1. The first element of the beam line is the white-beam slit. This slit is used to limit the vertical divergence, to exclude scattered radiation, and to control the heat load on the first monochromator crystal. The next element is the silicon carbide rhodium-coated spherical collimating mirror. The mirror is designed to vertically collimate the radiation in order to better match the inherent SR vertical divergence to the acceptance of the monochromator, but it has little effect on the horizontal beam divergence. We are now planning to install two mirrors, the collimating mirror and a flat mirror, so that they can be used alternatively. Certain crystals cannot take the heat and radiation load of the beam without thermal expansion and signal distortion. The flat mirror absorbs a large fraction of the beam energy and reduces the heat load as well as eliminating higher harmonics. The next element is the white-beam monitor, which operates by detecting the photoelectrons emitted from a tungsten mesh (90% transmittance). In combination with the white-beam slit, this monitor can be used to detect position shifts of the electron beam in the storage ring. Downstream of this monitor is the NSLS boomerang-type double flat crystal monochromator. After leaving the monochromator, the diffracted x-ray beam from the crystals is sampled by the channel electronic multiplier (CEM) monitor. Following the CEM monitor is a cylindrical aluminum refocusing mirror, which is electroless nickel plated and overcoated with rhodium. This mirror is designed to focus the beam at the sample to a diameter of about 1 mm. The next elements are the vertical and horizontal hutch slits located just upstream of the hutch. These slits can also be used to improve the energy resolution and to limit the size of the beam to match the sample size. The following element is the photon shutter, used to stop radiation from going into the hutch. Use of the photon shutter allows other upstream elements to remain illuminated, thus avoiding thermal transients and thereby improving the performance

stability of the beam line. The beam line is maintained at ultrahigh vacuum UHV up to the 10-mil thick beryllium window located inside the hutch.

TECHNICAL PROGRESS AND RESULTS: The performance of the X-19A beam line is largely determined by the choice of monochromator crystals. The monochromator has two presettable Bragg angle ranges: 8° to 15.5° and 14.97° to 69.14°. With Si(111) crystals, these angular ranges correspond to two energy ranges of $2.12 < E < 7.93$ keV and $7.4 < E < 13.53$ keV, respectively. The energy range can be extended to 22.10 keV with Si(220) crystals. Speciation analysis of S,K,Ca, and Fe compounds have been accomplished with the Si(111) crystals and Si(220) crystals. As can be seen from Figure 2, the soft x-ray capability of this beam line can be extended by using large d-spacing crystals such as InSb, Yb_{66}, and mica. An InSb crystal and a mica crystal have been obtained and will be tested.

As is shown in Figure 3, there is a considerable advantage in transmission by replacing the existing 10-mil Be window with a thinner one. Recent activity has centered on obtaining and installing a thin diamond film window, 0.4 μm thick. The purpose of this window is to be able to study elements as light as sodium and to improve the sensitivity for elements now being studied.

The originally-designed X-19A beam line system could speciate compounds of elements present in about the 100-ppm concentration range. The diamond window should provide a factor of four advantage by increasing the incoming flux. Much lower concentration ranges can be reached by replacing the single element detector commonly used by a multi-element detector. A 100-element detector is under development which should provide much higher sensitivities.

PAPERS/JOURNALS/PUBLICATIONS:

1. "The Effects of Biodegradation and Water Washing on Sulfur Compound Speciation in Crude Oils from the Bolivar Coastal Fields," Manowitz, B. and Jeon, Y., Accepted by ACS National Meeting, San Francisco, CA April 5-10, 1992. BNL-46518.

2. "Sulfur Compound Speciation in Sediments from the Peru Upwelling Region," Eglinton,T., Manowitz, B., Vairavamurthy, A., Jeon, Y., in preparation.

3. "Biochemical Reaction of Coal," Lin, Mow S., Manowitz, B. and Premuzic, E.T., Accepted by the Third International Symposium on the Biological Processing of Coal, Clearwater, Fla., May 4-7, 1992. BNL-47367.

4. "Oxidation State of Sulfur in Thiosulfate and Implications for Anaerobic Energy Metabolism," Vairavamurthy, A., Manowitz, B., and Jeon, Y., submitted to Nature, March 1992.

FOLLOW-ON FUNDING:

1. "Geochemical Incorporation of Sulfur into Organic Matter; Role of Polysulfides in the Formation of Organic Sulfur and the Preservation of Organic Matter during Early Diagenesis in Marine Sediments," A. Vairavamurthy and B. Manowitz;

funded in FY92 at $150,000 (B&R KC-04-03-02) operating and $25,000 capital.

2. Collaboration with Woods Hole Oceanographic Institution program entitled "Role of Sulfur in the Formation and Diagenesis of Macromolecular Matter in Sediments," B. Manowitz, Y. Jeon and T. Eglinton; funded in FY92 at $25,000 (B&R KC-04-03-02).

NOTE: This project does not involve human subjects. This project does not involve vertebrate animals.

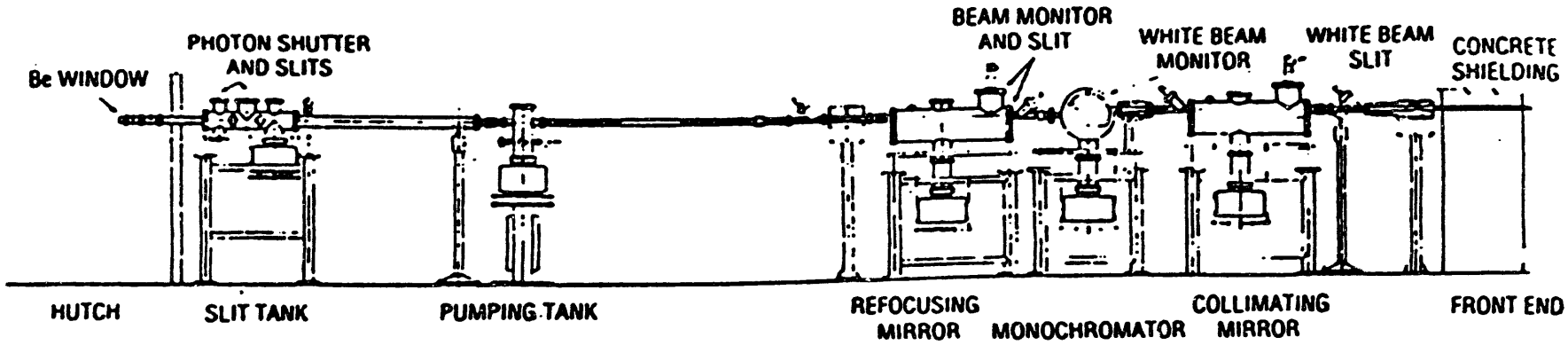
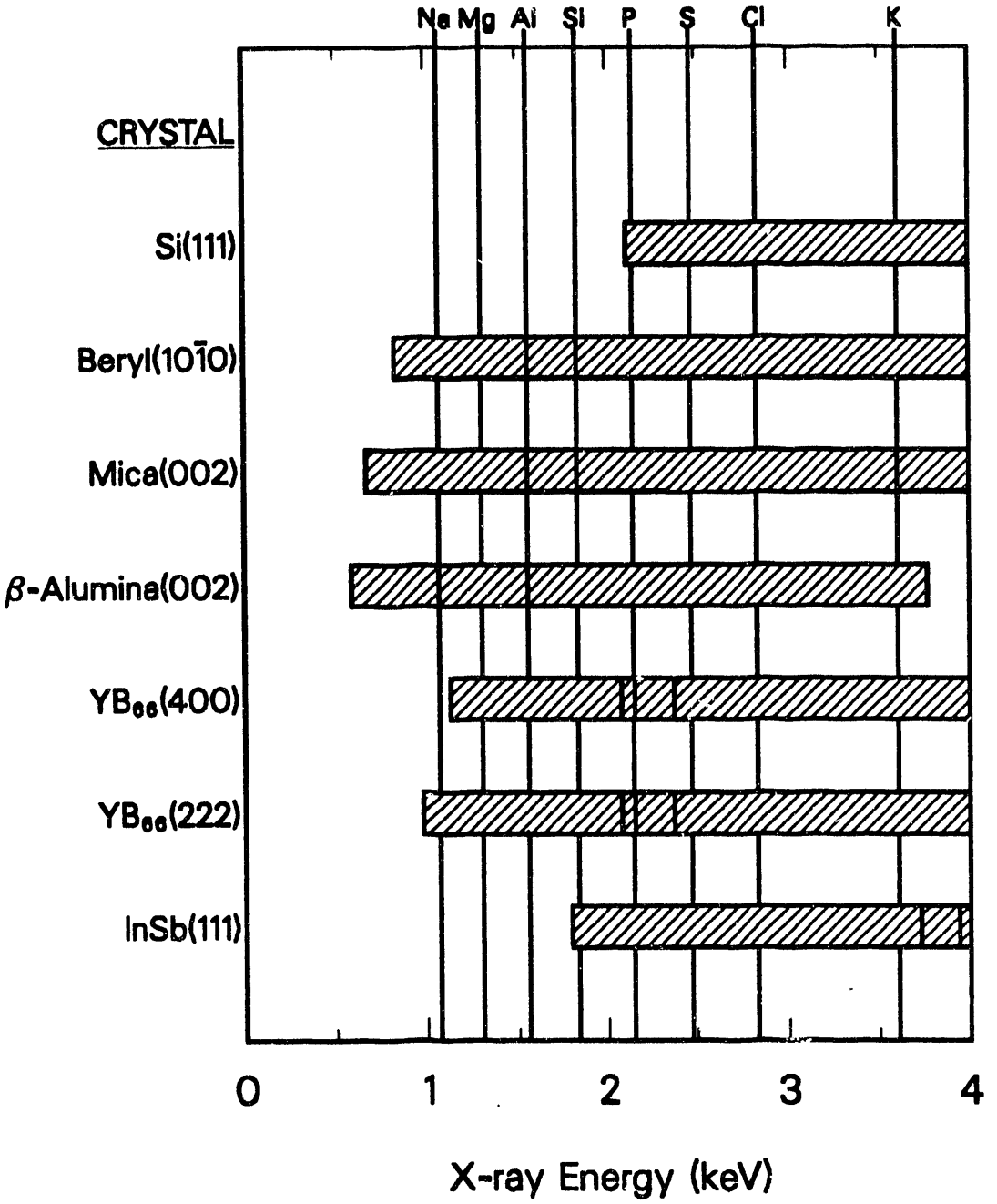
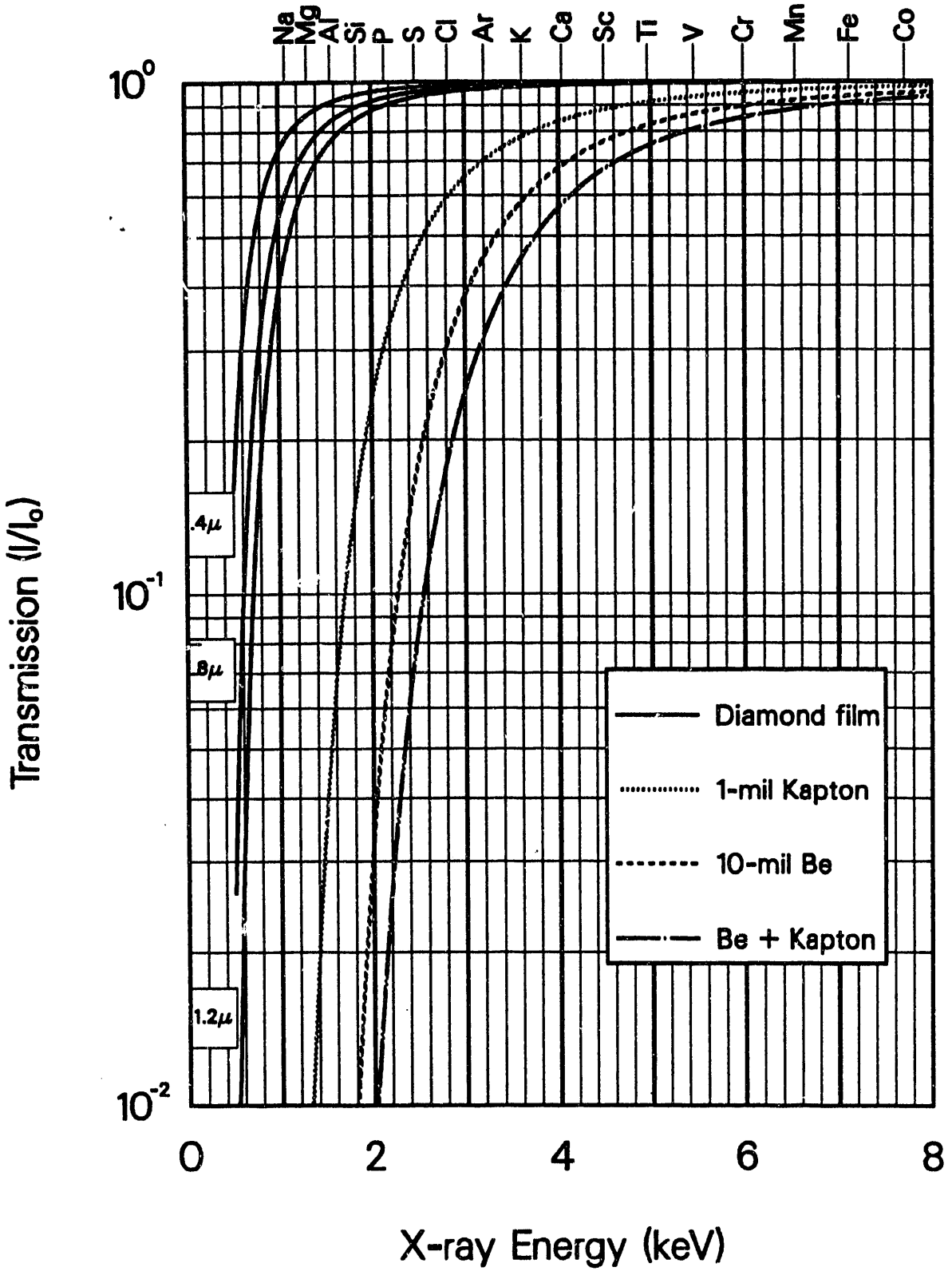


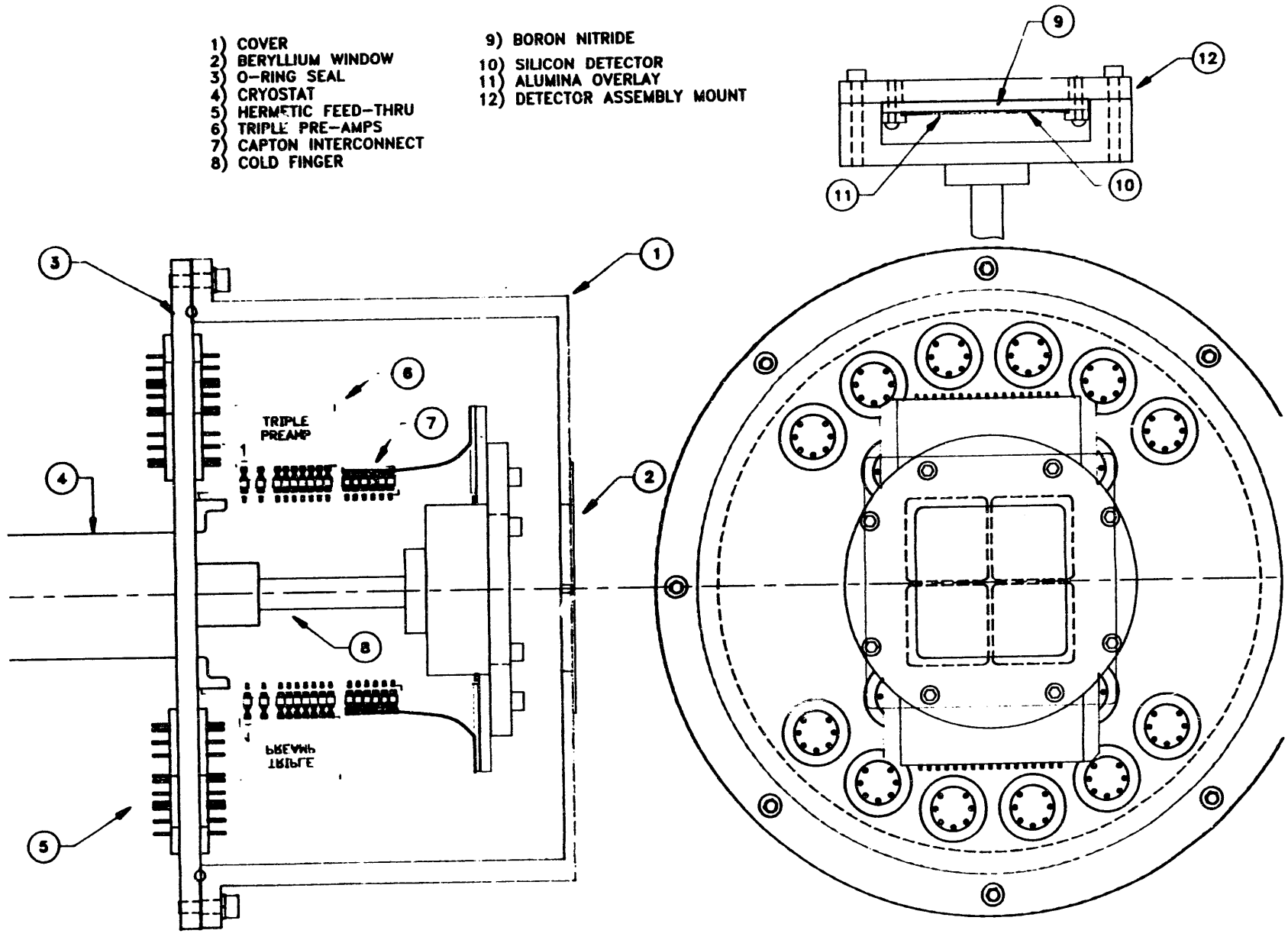
Figure 1. Layout of the key components of the X-19A beam line at the NSLS.

SOFT X-RAY MONOCHROMATOR CRYSTALS



WINDOW TRANSMISSION




1) COVER
2) BERYLLIUM WINDOW
3) O-RING SEAL
4) CRYOSTAT
5) HERMETIC FEED-THRU
6) TRIPLE PRE-AMPS
7) CAPTON INTERCONNECT
8) COLD FINGER
9) BORON NITRIDE
10) SILICON DETECTOR
11) ALUMINA OVERLAY
12) DETECTOR ASSEMBLY MOUNT
TRIPLE PREAMP

PROJECT NUMBER: 90-44

PROJECT TITLE: Transvenous Coronary Angiography with Synchrotron X-Rays

PRINCIPAL INVESTIGATOR: Thomlinson, W.

LDRD FUNDING:

FISCAL YEAR	AMOUNT
1990	$ 41,070
1991	29,043
1992	1,718
1993 (authorized)	0
1994 (estimated)	0
TOTAL	$ 71,831

PROJECT DESCRIPTION:

The unsolved problems of research related to ischemic heart disease are many. However, a large amount of new and significant information could be obtained if it were possible to noninvasively image the coronary arteries in man. Recently, a system using synchrotron generated x-rays and silicon detectors located at the Stanford Synchrotron Radiation Laboratory (SSRL), has demonstrated the principles of Iodine k-edge dichromagraphy in phantoms, anesthetized dogs, and human subjects. The Stanford studies confirm that transvenous coronary angiography is feasible with synchrotron radiation as the illuminating source. However, these studies also indicate that beams of greater x-ray intensity must be employed. The present proposal is a continuation of the original Stanford research utilizing the National Synchrotron Light Source (NSLS). Since NSLS can generate more x-ray fluence and a new dual-detector system will be utilized, an increase in available x-ray flux can be achieved over what was feasible at SSRL. The intensity available at NSLS should provide sufficient statistical accuracy to allow recording of high quality coronary angiograms. The sensitivity and specificity of the NSLS system will be ascertained in a group of patients who have undergone conventional coronary angiography. In another group of patients receiving medical therapy designed to limit or reduce atherosclerotic plaque mass, sequential coronary artery anatomy will be assessed. The angiographic facility becoming available at NSLS is the only one of its kind in the world and represents a unique opportunity for cardiovascular research.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: The goal of the LDRD for Transvenous Coronary Angiography with Synchrotron X-Rays was to demonstrate the capability of producing clinical research quality images of human coronary anatomy following venous injection of contrast agent. Throughout the project's lifetime, it was necessary to alternate human studies with advance instrumentation development. Such a process is necessarily very long and arduous. The resulting images with the previously existing system are very good, but not good enough to yet qualify for clinical evaluation. The only remaining problem appears to be that the photon flux from the X17 beamline is a factor of about 3 too small.

APPROACH: Since February of 1992, the NSLS has carried out an extremely aggressive program to construct, test, and commission a new dual energy Laue monochromator for the angiography work. The instrument was invented by W. Thomlinson (NSLS) and P. Suortti (formerly at University of Helsinki, presently at ESRF, Grenoble). Prototype testing began in February, with a final instrument tested for the first time in June 1992. The resulting photon flux is a factor of 12.6 times higher than the instrument previously used for the angiography imaging. A final test, prior to human studies, was done on beamline X17 from August 5-12. In parallel with this effort, the BNL Human Studies Review Committee (HSRC) approved operation of human studies with the new higher flux (radiation dose) levels. The radiation dose limit per single frame image was set at 7.0 rad/frame. No change was requested or granted, however, in the total dose limit per patient session which was kept at 35 rad.

TECHNICAL PROGRESS AND RESULTS: The project achieved a very significant goal of producing research quality images on Oct. 2, 1992. A 60 year old male from North Shore University Hospital who has had two myocardial infarctions was successfully imaged in SMERF. He was the eighth patient to be imaged under the LDRD project support. The new Laue monochromator was used for the first time. Images were obtained following venous injection of contrast agent. The dose was 5.4 rad/frame, a factor of about 8 higher than previous images. The resulting images in a LAO 45 degree projection showed the complete Right Coronary Artery, with excellent contrast and spatial resolution. Images in a RAO 30 degree projection revealed a totally occluded Left Anterior Descending coronary artery with a large collateral branch. The patient has been scheduled for a conventional angiogram in the near future. This patient weighed about 170 pounds, illustrating that the program can achieve excellent images with reasonably large individuals. In addition, the images have high enough signal to noise that absolute iodine images have been made allowing quantitative analysis and display of arterial cross sections.

Based in part on these successful images and those obtained in previous imaging runs supported by this LDRD project, funding has been obtained from DARPA (Defense Advanced Research Projects Agency) for a $1 M hardware development project. This will support the design and construction of the next generation imaging hardware to allow reliable long term research to commence. The DOE through the Office of Health and Environmental Research has also decided to support NSLS operations for the medical research programs. The success of the LDRD supported angiography project played a major role in those decisions. Research proposals will now be prepared for submission to the NIH.

PAPERS/JOURNALS/PUBLICATIONS:

1. "Venous Synchrotron Coronary Angiography," W. Thomlinson, N. Gmur, D. Chapman, R. Garrett, N. Lazarz, J. Morrison, P. Reiser, V. Padmanabhan, L. Ong, S. Green, A. Thompson, H. Zeman, R. Hofstadter, G. Brown, J. Giacomini, H. Gordon and E. Rubenstein, The LANCET 337, (1991) 360, BNL-46030.

2. "First Operation of the Medical Research Facility at the NSLS for Coronary Angiography,"

W. Thomlinson, N. Gmur, D. Chapman, N. Lazarz, R. Garrett, H. Moulin, A.C. Thompson, H.D. Zeman, G.S. Brown, J. Morrison, P. Reiser, V. Padmanabhan, L. Ong, S. Green, J. Giacomini, H. Gordon and E. Rubenstein, Rev. Sci. Instr. 63, (1992) 625, BNL-46109.

3. "Medical Applications of Synchrotron Radiation," W. Thomlinson, Nuc. Instr. and Meth. A319 (1992) 295-304, BNL-46865.

FOLLOW-ON FUNDING:

1. DARPA Proposal Submitted Oct. 1992 for $1,000,000 for hardware development (PIQ 92-10-2).

2. DOE-OHER proposes to support NSLS in its medical research programs. Amount to be determined.

NOTE: This project does involve human subjects. This project does not involve vertebrate animals.

PROJECT NUMBER: 90-45

PROJECT TITLE: Borehole Measurements of Global Warming

PRINCIPAL INVESTIGATOR: Hendrey, G.

LDRD FUNDING:

FISCAL YEAR		AMOUNT
1990		$ 88
1991		34,913
1992		49,518
1993	(authorized)	0
1994	(estimated)	0
TOTAL		$ 84,519

PROJECT DESCRIPTION:

This research addresses an hypothesis that global warming can be measured directly. A putative mechanism driving warming is the observed increase in atmospheric CO_2. This driver is strongly regulated by CO_2 exchange with the terrestrial environment due to photosynthesis, respiration, and gains or losses from terrestrial biomass, and soil carbon. Such changes will modify the surface radiative properties and, therefore, heat flux from the surface and propagation of temperature signals that may be "recorded" in borehole measurements. We are investigating the links in this hypothetical chain initially by concentrating on understanding photosynthetic mechanisms regulating carbon uptake in a CO_2-enriched environment potentially leading to a biomass change, thereby, strengthening the theoretical understanding of borehole measurements. Work in FY 1992 has concentrated on the first stage in the hypothetical chain, assimilation of CO_2 from the atmosphere in photosynthesis.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: The objective of the work conducted in FY 1992 has been to examine the extent and mechanisms by which photosynthesis, the process by which vegetation sequesters carbon from the atmosphere, is modified by growth in elevated CO_2 atmospheres. There is a good understanding of how and why photosynthesis in terrestrial C_3 plants, grown in the current atmosphere, respond to instantaneous increases in CO_2 concentration. However, ability to predict future rates of sequestration of carbon from the atmosphere by photosynthesis is impeded by lack of understanding of the mechanisms by which photosynthetic capacity is itself affected by atmospheric change. Rising concentrations of CO_2 and O_3 in the troposphere alter plant development and in particular may decrease photosynthetic capacity. This represents a potential negative feedback in the capacity of vegetation to remove carbon from the atmosphere and ameliorate the rise in CO_2 concentrations and associated global warming. Work in FY 1992 has concentrated on understanding of these changes in photosynthetic capacity and its incorporation into a mathematical model of vegetation response.

APPROACH: The strategy for FY 1992 has been a) to establish a physiological and

molecular capability for examining mechanisms of photosynthetic acclimation to rising CO_2 concentrations; b) to utilize plant material grown in DOE funded long-term studies of elevated CO_2 concentration to examine changes at the protein level; c) to test hypotheses developed under b in controlled environments; and d) to incorporate understanding developed from b and c into the further development of a mathematical model of photosynthetic CO_2 uptake to examine the implications of our findings in a wider context.

TECHNICAL PROGRESS AND RESULTS:

1. Facilities have been established or extended for: a) the in vivo physiological analysis of limitations to photosynthesis; b) the analysis of polypeptide and RNA levels in terrestrial plant leaves; c) controlled environment growth in varying CO_2 concentrations and at realistic light fluxes; and d) mechanistic mathematical modeling of photosynthetic CO_2 uptake in response to atmospheric and climate change.

2. Previous studies had shown decreases in the protein Ribulose 1,5- bisphosphate carboxylase/oxygenase (RuBisCo, the primary carboxylase of C_3 photosynthesis) in response to long-term exposure to elevated CO_2. Our analyses of Scirpus olneyii (Chesapeake Bay, MD) and Orange (Phoenix, AZ), both grown for 6 years at double current atmospheric CO_2 concentrations, show that not only Rubisco but many other soluble leaf proteins are significantly decreased - suggesting that decreased photosynthetic capacity is not simply a result of decreased RuBisCo, as previously suggested. We are currently working to identify these proteins. By contrast, no changes in any of the thylakoid proteins were observed.

3. Photoinhibition of photosynthesis is a common event in the field during exposure of plants of full sunlight. This leads to a temporary impairment of capacity for CO_2 assimilation which could depress CO_2 uptake by 10 - 50%. By in vivo analysis we have examined the hypothesis that accumulation of carbohydrates in leaves grown at elevated CO_2 concentrations may exacerbate photoinhibition. Investigating Cotton, Scirpus, and Orange grown in the field, and Wheat and Cotton grown in high light controlled environments, we found no instance in which photoinhibition was increased by growth in elevated CO_2 concentrations. In contrast,in July in Scirpus and in September in Orange, in the field, photoinhibition was significantly less and not more in the plants growing in elevated CO_2 concentrations. The results suggest, in contrast to expectations, that elevated CO_2 concentration does not appear to exacerbate photoinhibition,and in some circumstances it may protect against photoinhibition. Possible mechanisms of this protection are the subject of current work. These data will be used in the context of the model to determine the potential positive feedback on CO_2 uptake that this protection could represent.

4. Incorporation of observations of decreased RuBisCo concentrations into our model suggest that at low air temperatures this would lead to decreased rates of photosynthetic CO_2 uptake in a doubled CO_2 atmosphere, despite suppression of photorespiratory losses of C. This hypothesis was tested by growing Wheat of the cultivar chosen for the upcoming FACE-Wheat experiment in controlled environments with 14C days and 10C nights in current and doubled CO_2 atmospheres. Although, growth at low temperatures negated any stimulation of photosynthesis in a doubled CO_2 atmosphere, the plants grown

and measured in the doubled CO_2 atmosphere did not show a significantly lower rate of CO_2 uptake by comparison to the plants grown and measured in the current CO_2 atmosphere. This suggests that the predicted negative feedback effect of elevated CO_2 in cold environments may not occur. This will be further tested in the early growth stages of Wheat in the FACE experiment starting this Fall.

5. Incorporation of changes in RuBisCo activity induced by growth in elevated CO_2 concentrations suggests, that despite this loss of a commonly limiting enzyme, photosynthetic CO_2 uptake at temperatures above 15 - 20C would still exceed those for C_3 plants growing at current concentrations. However, O_3 also depresses leaf RuBisCo levels and activities - if current trends of increase in tropospheric O_3 concentrations in concert with rising CO_2 concentrations continue, then decreases in RuBisCo would be such that photosynthetic rates of CO_2 uptake would be depressed at most temperatures. Further work has been proposed to examine this potential interaction in depressing the capacity of vegetation to ameliorate the rise in atmospheric CO_2 concentrations.

PAPERS/JOURNALS/PUBLICATIONS:

1. LONG, S.P., NIE, G.-Y., DRAKE, B.G., FARAGE, P.K., HENDREY, G.R. & LEWIN, K.H.(1992) The implications of concurrent increases in temperature, CO_2 for terrestrial C_3 photosynthesis. In: Proc. IXth Int. Congr. Photosyn Res (Murata, N., ed.) Kluwer Academic, Dordrecht (in press).

2. NIE, G.-Y. & LONG, S.P. (1992) The effect of prolonged growth in elevated CO_2 concentrations in the field on the amounts of different leaf proteins. In: Proc. IXth Int. Congr. Photosyn. Res. (Murata, N., ed.), Kluwer Academic, Dordrecht (in press).

3. LONG, S.P. (1992) The potential effects of concurrent increases in temperature, CO_2 and O_3 on net photosynthesis, as mediated by RuBisCo. In: Proceedings of third international symposium on Gaseous Pollutants and Plant Metabolism (R. Alscher & A.J. Wellburn, eds.), Elsevier Applied Sci, London (in press).

4. LONG, S.P. (1992). Resource capture by leaves. In: Proc. 52nd Easter School in Agricultural Science (J.L. Montheith, K.K. Scott and M.H. Unsworth, eds.), Butterworths, London (in press).

5. LONG, S.P., POSTL, W.F. & BOLHAR-NORDENKAMPF, H.R. (1992) Quantum yields for CO_2 uptake in C_3 vascular plants of contrasting habitats and taxonomic groupings. Planta (in press).

6. LONG, S.P., BAKER, N.R. & RAINES, C.A. (1992) Analyzing the responses of photosynthetic CO_2 assimilation to long-term elevation of atmospheric CO_2 concentration. Vegetatio (in press).

NOTE: This project does not involve human subjects. This project does not involve invertebrate animals.

PROJECT NUMBER: 91-03

PROJECT TITLE: Molecular Ecology: Development of Field Methods for Microbial Growth Rate and Activity Measurements

PRINCIPAL INVESTIGATOR: Kemp, P.F.

LDRD FUNDING:

FISCAL YEAR		AMOUNT
1990		$ 0
1991		49,229
1992		50,602
1993	(authorized)	0
1994	(estimated)	0
TOTAL		$ 99,831

PROJECT DESCRIPTION:

DOE research on bioremediation of contaminated ground-water has focused strongly on bacterial growth and activity, which are known to drive major biogeochemical cycles including the transformation and degradation of many contaminants. However, existing methodologies for measuring bacterial growth and activity have severe limitations including a lack of specificity, low signal/background ratio, interference from sediment particles, and sample handling artifacrts. It is proposed to develop methods to measure in-situ, species-specific bacterial growth rate and enzyme synthesis, using molecular biology techniques which address these limitations. The proposed approach is a major step toward understanding subsurface microbial ecology, and is broadly applicable to other natural ecosystems.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: Existing methodologies for measuring bacterial growth and activity in marine, freshwater and groundwater environments have severe limitations. These include a lack of specificity, low signal/background ratio, interference from sediment particles, and sample hardening artifacts. This project was designed to provide working field methods for measuring growth rates using molecular biology techniques which address these limitations. Specifically, we are developing procedures for measuring cell-specific ribosomal RNA content, which is a primary determinant of growth rate, and using these data to predict the instantaneous specific growth rate. The approach provides growth rate estimates which are taxon-specific, cell-specific, and incubation-free.

An ideal method for measuring bacterial growth should:
a) have no influence on the rates to be measured,
b) be capable of distinguishing among targeted and non-targeted microbes,
c) permit measurement of very low growth rates and growth of relatively rare cells,
d) measure growth in situ, and
e) be immune to interference from a soil/sediment matrix.
Existing methods fail to meet several of these basic requirements, whereas the

methods we are developing can potentially meet all requirements.

APPROACH: We are utilizing molecular-biology techniques to meet these criteria for the measurement of in-situ bacterial growth rates in aquatic and soil/sediment environments. The approach taken at BNL has been based on the work of DeLong, et al. (1989) with fluorescently-labeled, oligonucleotide hybridization probes. The strength of this approach is that one can obtain cell-specific, incubation-free, and taxon-specific estimates of rRNA content. Probes are complementary to 16S rRNA sequences, and are labeled by conjugation with the fluorochrome Texas Red. When hybridized to 16S rRNA in preserved, intact cells, the resulting cell fluorescence is directly proportional to rRNA content and therefore in principle will be correlated to growth rate. Probe sequences can be selected which are characteristic of specific phylogenetic groups (Giovannoni, et al. 1988), ranging from universal probes, to kingdom-level (eubacteria, archaebacteria, or eukaryotes), to extremely specific probes (DeLong, et al. 1989; Gobel, et al. 1987; Salama, et al.. 1991). Therefore, one can use specific probes to measure the rRNA content of single species within a mixed bacterial assemblage and from this infer its growth rate independently of other co-occurring species. Because cells are examined individually, the frequency distribution of growth rate among cells also can be determined and relatively active and inactive cells can be distinguished. Typical frequency distributions of rRNA-specific fluorescence shift in a predictable manner to lower RRNA content at slower growth rates, higher RRNA contents at faster growth rates. This provides an extremely useful indicator of population response to environmental change.

TECHNICAL PROGRESS AND RESULTS: We developed a working system for measuring bacterial RNA and DNA in individual cells, using a scanning microphotometer mounted on a Mikon Optiphot epifluorescence microscope. These measurements were calibrated in the laboratory against fluorometric, bulk-extract assays of RNA and DNA in single-species cultures under a variety of controlled growth conditions. We tested the laboratory-based calibration curve by repeated sampling of mixed field populations of bacteria. RNA content estimates based on the probe-fluorescence method consistently match estimates based on bulk extractions of field samples.

We have conducted batch and continuous-culture (chemostat) experiments to empirically test the relationship between RNA content and growth rate. We find that RNA:growth rate regressions are specific to bacterial species, but for any one species the relationship is extremely strong. On the average, 96% of variation in chemostat data is explained by species-specific regressions. Even ignoring species differences, RNA content is a better generic predictor of growth rate than other available methods. We have found that the RNA:growth rate relationship exists for all tested species under equilibrium conditions, but only for some under rapidly-changing batch-culture conditions.

We developed a signal-enhancement method that utilizes a mixture of oligonucleotide probes which have equivalent specificity and similar fluorescence yields. With this mixture, we can examine the seasonal variation in RNA/cell in field samples. We are now in the 9th month of a monthly sampling program, in which changes in bacterial RNA content, size, and growth rate are followed at a Great South Bay site.

Finally, we initiated experiments with marine protists which explore the relationship between their RNA content and growth rate in natural seawater culture. This work will be continued in collaboration with visiting students and scientists during the next year.

PAPERS/JOURNALS/PUBLICATIONS:

1. Kemp, P.F., S. Lee, J. LaRoche. Estimating the growth rate of marine bacteria from cellular RNA content. Appl. Environ. Microb., to be submitted in October 1992.

2. Kemp, P.F., S. Lee, J. LaRoche. Estimating the growth rate of marine bacteria from ribosomal RNA content measured with 16S rRNA-targeted oligonucleotide probes. Appl. Environ. Microb., in preparation.

3. Lee, S., C. Malone, and P.F. Kemp. Use of multiple 16S rRNA-targeted fluorescent probes to increase signals and measure cellular RNA from natural planktonic bacteria. Submitted to Appl. Environ. Microb., submitted.

FOLLOW-ON FUNDING:

1. Measurement of marine microbial growth. Proposal to DOE Marine Program (Ocean Margins Program) funded at $214,000 in FY 1993 (B&R KP-02-02).

NOTE: This project does not involve human subjects. This project does not involve vertebrate animals.

PROJECT NUMBER: 91-04

PROJECT TITLE: A New Malaria Enzyme - A Potential Source for a New Diagnostic Test for Malaria and a Target for a New Antimalarial Drug

PRINCIPAL INVESTIGATOR: Mangel, Walter F.

LDRD FUNDING:

FISCAL YEAR		AMOUNT
1990		$ 0
1991		52,763
1992		55,875
1993	(authorized)	59,000
1994	(estimated)	0
TOTAL		$167,638

PROJECT DESCRIPTION:

It has been observed with a fluorogenic substrate a highly specific proteinase activity on the surface of Plasmodium falciparum during infection of human red blood cells. It is proposed to develop a highly sensitive test for malaria infection based upon an assay of this enzyme with a fluoregenic substrate. It is also proposed to synthesize an inhibitor of this enzyme to be used as an antimalarial drug.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: As classical drug treatment of malaria becomes less efficient because of resistant parasites and with the difficulties in producing effective vaccines, the development of new antimalarial agents becomes increasingly important. Our objective is to test the hypothesis that a highly specific proteinase whose activity is required for reinvasion of erythrocytes by P.falciparum is a legitimate target for rationally designed drugs. The hypothesis will be tested by synthesizing equally specific proteinase inhibitors that should inhibit reinvasion but not normal physiological processes.

APPROACH: We have obtained preliminary evidence that this proteinase is located on the cell surface of merozoites and is irreversibly inhibited by bovine pancreatic trypsin inhibitor (BPTI). Based upon its affinity for BPTI, the proteinase will be purified and its gene cloned and expressed in E.coli. The substrate specificity of the recombinant proteinase will be determined, and the optimal substrate sequence incorporated into the reactive site of BPTI. The resultant mutant BPTI should be a highly specific inhibitor of this proteinase and no others. There are good experimental systems within which to measure the efficiency of BPTI and mutants of BPTI, from in vitro studies on the inhibition of the recombinant proteinase, to curing culture of red blood cells infected by P.falciparum to curing a mouse infected by P.chabaudi.

TECHNICAL PROGRESS AND RESULTS: Initially we incubated infected red blood cells with the fluorogenic substrate (Cbz-Ile-Pro-Arg-$NH)_2$-Rhodamine and saw the parasite become fluorescent in a fluorescence microscope. The reaction is

specific as we tried other substrates such as (Cbz-Phe-Arg-NH)$_2$-Rhodamine and (< Gly-Gly-Are-NH)$_2$-Rhodamine and saw no fluorescence. We could inhibit the fluorescence generated by the presence of (Cbz-Ile-Pro-Arg-NH)$_2$-Rhodamine with BPTI but not other serine proteinase inhibitors including NPGB. Most important, we were able to inhibit reinvasion of erythrocytes by the presence of BPTI. Thus all the preliminary evidence we have obtained so far indicates that we have discovered a trypsin-like proteinase on the surface of the parasite whose activity is required for reinvasion of erythrocytes.

PAPERS/JOURNALS/PUBLICATIONS:

1. Barale, J.-C., Langsley, G., Mangel, W., and C. Braun-Breton. 1992. Malarial Proteases: Assignment of Function to Activity. Research in Immunology, 672-681.

NOTE: This project does not involve human subjects. This project does not involve vertebrate animals.

PROJECT NUMBER: 91-07

PROJECT TITLE: Basic Studies on Thoron and Thoron Precursors

PRINCIPAL INVESTIGATOR: Harbottle, G.

LDRD FUNDING:

FISCAL YEAR		AMOUNT
1990		$ 0
1991		37,941
1992		51,991
1993	(authorized)	0
1994	(estimated)	0
TOTAL		$ 89,932

PROJECT DESCRIPTION:

This project is concerned with the behavior of thoron and its precursors in the thorium-232 chain in soils. Both thoron and radon are health hazards, but the level of research on thoron, its health effects, transport in soil and air, entry into buildings, etc., is very low compared to that on radon. Thus, because of the greatly differing lifetimes, the radioactive flux of thoron that escapes from the earth's surface is greater than that of radon. It is clear that a better knowledge of the behavior of thoron and its precursors is crucial to evaluating the dimensions of the thoron problem. There is no reason to suppose that the relationships that govern the availability if radon in soils also necessarily apply to thoron.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: The background is as stated above; briefly, although thoron (Rn 220) is a health hazard generally thought to contribute 20-30% additional ionizing (alpha-particle) dose to the lung (relative, and in addition, to radon (Rn222) there has been very little research on its properties in soils. These include, the relationship between thoron flux and thorium content, the ease of escape (emanation coefficient or probability) from soils, and the determination of the state of secular equilibrium. It was the belief of the P.I. that, given the limited funding available for radon and thoron research, the DOE ought to focus its efforts in soil radioactivity on those relatively limited geographical portions of the USA where radionuclide content, soil permeability and emanation coefficients have combined to produce a high radon and thoron risk. The Appalachian Foldbelt, consisting of miogeosynclinal and eugeosynclinal deposits of Precambrian to Paleozoic age, Paleozoic granites and Precambrian metamorphic and plutonic rocks, is one such area. This foldbelt extends from the area of Atlanta, GA and Birmingham, AL on the south through the western Carolinas and eastern Tennessee, Virginia (Roanoke), Washington, D.C., eastern Pennsylvania and New Jersey up into Connecticut, Rhode Island, Massachusetts, Vermont, New Hampshire and Maine. To the P.I. these considerations were persuasive as to the choice of the general region of experimental interest. The specific choice within these high risk soils was the Conway granite region of New Hampshire. This was the choice because it was the research specialization area of the collaborating soil

scientist Professor Christine V. Evans of the University of New Hampshire at Durham. To quote Professor Evans: "The Conway granite occurs commonly in the White Mountains and is remarkable because it contains high (>50 ppm) levels of Th and 11-14 ppm U, which make the Conway granite roughly twice as radioactive as most silicic igneous and metamorphic rocks in North America. It is sufficiently radioactive to have provoked interest as a possible source of geothermal energy, and record-setting air radon levels have been recorded in a house located on Conway granite in northern New Hampshire." In discussions with Professor Evans, issues critical to the investigation of thoron sources in the soil were delineated, and one of the most important of these was the issue of weathering. The term "weathering" in pedology subsumes a whole range of natural events (including chemical, physical and biological processes) occurring to convert and modify the original rock into the final soil.

Dr. Evans supplied a series of about 30 soils which had already been pedologically characterized from four cross-sections, or "profiles" of Conway granite derived soils located on Mt. Major and Mt. Welch in New Hampshire.

These then were the primary goals of the experimental design of the present LDRD project:

- to characterize the important rock and soil weathering processes associated with the transport and/or retention of thorium in soils,
- to determine the role of various soil constituents (separated as necessary by chemical or physical fractionation) in providing sites for radionuclide accumulation, and
- to relate this knowledge through the measurement of the various radionuclides in soil fractions, and of the emanation coefficients of thoron in the same fractions, to the overall problem of thoron availability.

APPROACH (Experimental Methods): Because of the variety of natural materials to be studied in this research (the 30 soils comprised several soil types, top soil with organic matter, rock, saprolites, etc.) it seemed imperative that broadly-applicable, general methods and instrumentation first be developed. Therefore, the primary goal was to fabricate, assemble and calibrate equipment capable of precise, sensitive, quality-controlled quantitative nuclear gamma ray measurements of members of the natural radioisotopic thorium series at concentration levels existing in nature.

The capability of measuring natural radioisotopes in soils permits us to determine:

a) the analytical concentrations of thorium along with uranium and potassium, radium and other shorter-lived members of the natural decay chains;

b) the fraction of radon and thoron which can escape the soil grains (the "emanation fraction"); and

c) the departure from secular equilibrium (if any) existing in the radioisotope series found in the whole soils and in separated soil fractions.

Emphasis is placed on gamma ray counting to achieve the goals of this project because it is a generally applicable method with a minimum of operator, bench-chemistry intervention. The chemical analysis of soils via wet chemistry is slow and demanding and if employed here would greatly restrict sample throughput. This in turn would severely limit the ability to view soil radioactivity as a statistically-distributed

phenomenon. Also, when whole soils are measured, the observations relate to more natural samples whose characteristics can be more readily extrapolated to real environmental conditions.

TECHNICAL PROGRESS AND RESULTS: Considerable effort was placed on the procurement of sensitive gamma detectors and the design of counting cells for containing the soil. A number of counting cells of the "Marinelli" design were fabricated locally from metal and glass and tried, as well as commercially-available plastic models. From economic considerations, it was evident that commercially-produced cells had to be employed for routine measurements. It was found that most of these performed satisfactorily in making relative gamma ray counts of materials having approximately the same density and composition, but failed when an attempt was made to model their gamma ray self-absorption so that the absolute radionuclide concentrations could be determined in a variety of materials (rock, clays, soils, saprolites, etc.) of differing density and composition.

The solution to this problem was the development of a cell that was commercially available and was modified at BNL to provide a container that could be successfully mathematically modeled. The creation of software to do this and the calibration of the cell with commercial standards (traceable to NIST) and in-house standards is now complete. The modified cell contains about 600 g. of typical soil. Quality control measures involved calibration with known NIST, or NIST-traceable, CANMET, DOE and IAEA standards.

The rationale for the choice of the New Hampshire Conway granite soils is presented above. Samples of all the soil horizons in three separate soil profiles on Mt. Major have now been counted at high precision using the modified Marinelli cells described above and the data reduced to uranium, thorium and radium concentrations, values of the disequilibrium parameters for uranium and thorium series and potassium content via K40 measurement.

The results are presented (without a detailed explanation of their meaning) in the table entitled "Report" at the end of the software printout, appended. Emanation coefficients were also measured.

For a number of these horizons, fine fractions have been separated by wet or dry sieving of the soils and the radionuclides determined separately. These samples are labeled "F" under "Type" in the "Report" section.

Although a complex picture of soil-radionuclide interactions is revealed, certain trends are fairly evident. Conway granite from deep cores, when powdered emanates very little radon or thoron, and is in secular equilibrium. More shallowly buried (i.e. a few meters) rock or grus, although they are not desegregated are generally capable of emanating thoron freely. The soil column as a whole including the spodic horizon is deficient in Ra226. Spodic horizons, however, are highly enriched in uranium and thorium while A horizons are deficient in U and Th.

From the perspective of the goal of this LDRD project, the study of thorium in soils, its translocation following mobilization by weathering and the effect this has on thoron availability, one of the most significant findings is the startling enrichment of thorium during weathering. From Conway granite unaltered rock levels of ca. 40-50

ppm thorium is enriched in the fine fractions of some C-horizon soils to astonishingly high levels, 150-175 ppm. From such fractions thoron can emanate with a probability of 20-40%. Additional experiments are planned to relate these enriching processes to the chemistry of rock weathering.

PAPERS/JOURNALS/PUBLICATIONS:

1. W.-J. Lin and G. Harbottle "Gamma Ray Emission Intensities of the 232Th Chain in Secular Equilibrium," J. Radioanalytical Nucl. Chem, vol 157, 367 (1992).

2. W.-J. Lin and G. Harbottle "Determination of Diffusion Lengths and Flux Densities of Radon and Thoron in Soils" Health Physics (submitted).

FOLLOW-ON FUNDING:

$189,000 from DOE-ER in FY 1993 (B&R KP-02-03).

NOTE: This project does not involve human subjects. This project does not involve vertebrate animals.

PROJECT NUMBER: 91-08

PROJECT TITLE: Cloning of the cDNA for a Human Serine/Threonine Protein Kinase that is Activated Specifically by Double-Stranded DNA

PRINCIPAL INVESTIGATOR: Anderson, C.W.

LDRD FUNDING:

FISCAL YEAR		AMOUNT
1990		$ 0
1991		85,318
1992		89,466
1993	(authorized)	0
1994	(estimated)	0
TOTAL		$174,784

PROJECT DESCRIPTION:

Gene expression in human cells is regulated by proteins that bind to specific DNA sequences. The interaction of these regulatory proteins with DNA is, in turn, controlled by extra- and intracellular signals that, in many cases, cause protein phosphorylation. We have identified a nuclear protein kinase from human cells that is strongly activated by double-stranded DNA. This dsDNA-activated protein kinase phosphorylates several regulatory proteins including the large tumor antigen (TAg) of simian virus 40 (an oncogene product), the product of the mouse tumor suppressor gene p53, topoisomerases I and II, the progesterone receptor, and the transcription factors Spl, Oct-1, and Oct-2, and C/EBP. The results suggest that, like the cell cycle regulated cdc2/p34 protein kinase, the dsDNA-kinase has a major role in regulating transcription, DNA replication, and/or cell growth. The purpose of this proposal is to identify the gene for the human dsDNA-activated kinase and to identify the amino acids in the Oct transcription factors that are phosphorylated by this dsDNA-kinase.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: Protein phosphorylation is the most widely used post-translational mechanism for the control of cell processes and biochemical functions in eukaryotic cells. It controls progression through the cell cycle, chromatin structure, transcription of messenger RNAs, and translation of messenger RNAs into proteins. Forty percent of all human proteins are phosphoproteins; however, in only a few cases are the enzymes responsible for the addition (kinases) and removal (phosphatases) of phosphate known.

As a consequence of our research on adenovirus gene expression at Brookhaven, we found and partially purified a protein kinase from cultured human HeLa cells that is activated specifically by double-stranded DNA (dsDNA). We called this kinase DNA-PK. Few enzymes are known that are regulated by DNA; however, our finding suggests that protein kinases may function as monitors of DNA or chromatin state in animals cells. DNA-PK probably is present in the nucleus of most human cells, and closely related DNA-activated kinases

appear to be present in many multicellular animals. Our initial work suggested that the dsDNA-kinase regulates the life cycle of the small DNA tumor virus, simian virus 40 (SV40). Further study has shown that DNA-PK phosphorylates a number of cellular DNA binding proteins including factors that regulate transcription and DNA replication. These properties suggest that DNA-PK may be an important part of a cellular mechanism that detects DNA damage and/or cell division.

Using the protein sequencing facilities in the Biology Department, we identified the amino acids that can be phosphorylated by DNA-PK in vitro in several substrates. Phosphorylation occurs at two amino-terminal threonine (Thr) residues in the heat-shock protein hsp90. Four serine residue were identified as sites of DNA-PK phosphorylation in the large tumor antigen (TAg) of SV40. These studies suggested that DNA-PK may recognize serine or threonine residues in protein substrates that are followed immediately by glutamine residues. This hypothesis recently was confirmed for a DNA-PK phosphorylation site in the human tumor suppressor protein p53 and in the serum response transcription factor SRF. However, serines or threonines in other substrates that are not followed immediately by glutamine can be phosphorylated by DNA-PK. This result raises the possibility that cells may encode more than one DNA-activated protein kinase.

Purification and characterization of the DNA-PK activity from human tissue culture cells indicated that kinase activity was tightly associated with a 350-kilodalton (kDa) polypeptide; thus, this ~3500 amino acid/polypeptide is likely to be the principle subunit of DNA-PK enzyme. We also found that the 350-Kda polypeptide is phosphorylated in a DNA-dependent manner, and this "autophosphorylation" is associated with a rapid loss of kinase activity, suggesting that DNA-PK can turn itself off by self-phosphorylation.

The main objective of our proposal is to identify and select bacterial plasmid clones that have sequences corresponding to the messenger RNA for human DNA-PK. We also proposed to determine the sites that are phosphorylated by DNA-PK in the POU DNA-binding domain of the human transcription factor Oct-1. Cloning and preliminary sequence analysis of the DNA-PK cDNA is a collaboration between this laboratory and the laboratory of Dr. Steve Jackson, now at the CRC/Wellcome Research Institute, Cambridge, England.

Bacterial clones corresponding to the mRNA for the 350-kDa DNA-PK polypeptide are essential for many analyses that will contribute to identifying the function performed by DNA-PK in human cells. The nucleotide sequence of the cDNA will permit us to deduce the amino acid sequence of DNA-PK. These clones also would permit us to determine where and when the 350-Kda DNA-PK polypeptide is expressed, to determine if human cells have other genes related to DNA-PK, to identify the chromosome and chromosomal location of the DNA-PK gene, to determine if the gene is located near known human genetic disease loci, and to identify similar genes from other organisms. We believe the POU domain of the Oct-1 protein will be a useful substrate for developing an assay that will permit us to determine when DNA-PK is activated in human cells.

APPROACH: The 350-kDa DNA-PK polypeptide will be purified from cultured human HeLa cells, and the purified polypeptide will be digested into smaller

peptides for partial protein sequence analysis. Degenerate oligonucleotides that could encode segments of the sequenced peptides then will be synthesized and these will be used to identify cDNA clones in a library of clones prepared from reverse transcripts of the mRNAs present in human cells. The POU domain of the Oct-1 protein will be subcloned into a bacterial expression vector that will permit rapid purification of the unphosphorylated protein. After in vitro phosphorylation, the purified POU protein will be digested with appropriate enzymes, the radioactive peptide(s) containing the site(s) of phosphorylation will be separated from unphosphorylated peptides, and the sequence of the phosphopeptide(s) will be determined. If the phosphorylated peptide contains more than one serine or threonine, the precise residue phosphorylated will be identified by radiochemical sequencing or further chemical analysis.

TECHNICAL PROGRESS AND RESULTS: The 350-Kda DNA-PK polypeptide was purified from cultured human cells and digested to produce peptides. After fractionation by reverse-phase chromatography, selected peptides were sequenced. Sets of synthetic oligonucleotides that could encode selected peptides were made, and these were used to screen DNA clones (cDNA libraries) corresponding to human mRNAS. A screen of three cDNA libraries produced one clone containing a ~1200 base-pair (bp) cDNA insert; a second clone containing an overlapping ~1200 bp insert was obtained by re-screening the libraries with DNA from the first clone. Analysis of HeLa mRNA using these clones suggests that the DNA-PK mRNA is 12-14 kbp in length.

Sequence from the first clone was subcloned into an E. coli expression vector (pATH3) and the resulting fusion protein (from plasmid C8-11) was purified and used to immunize a rabbit. Hyperimmune serum from the rabbit reacted with a 350-kDa polypeptide in crude lysates of HeLa cells and with the 350-kDa polypeptide in a purified kinase preparation. This result indicates that the isolated cDNA fragments actually correspond to mRNA fragments for the 350-Kda polypeptide. Subsequently, two small regions (~100 amino acids each) of the cDNA sequence were subcloned into bacteriophage T7 expression plasmids that provide an epitope which facilitates purification of the expressed cDNA segment. The regions chosen for expression are predicted to be on the surface of the native DNA-PK polypeptide; thus, we anticipate that these antisera, unlike the anti-C8-11 antisera, may react with the native 350-kDa polypeptide. Analysis of the two new antisera will begin shortly.

Five bacteriophage lambda clones have been isolated, each of which contains different 15-20 kbp inserts of human genomic DNA that hybridizes with part of our ~3-kbp DNA-PK cDNA fragment. These clones are being analyzed to identify intron/exon boundaries. This information will be used to develop a quantitative assay for DNA-PK mRNA, for preparing probes for isolating the remainder of the DNA-PK cDNA, and for isolating the complete DNA-PK gene as yeast artificial chromosomes.

In collaboration with D. Ward, Yale University, the two ~1200-kbp cDNA clones were used to identify the location of the DNA-PK gene by in situ hybridization; the DNA-PK gene maps to band 11q of human chromosome 8. This region of chromosome 8 also encodes two other growth related protein kinases, the mos and lyn protooncogenes. The genetic defect responsible for Werner syndrome also has been mapped to this region of

chromosome 8. Werner syndrome is a rare, autosomal, recessive disease that is characterized by symptoms of premature aging and early death.

Through our DOE sponsored research, we recently developed a rapid, quantitative peptide assay that appears to be highly specific for DNA-PK. This assay was used to quantitate the amount of DNA-activatable kinase in normal skin fibroblasts and in fibroblasts derived from patients with Werner syndrome. Two Werner syndrome cultures had two to five fold less activatable DNA-PK than cultures from age sex match normal patients. This preliminary result suggests that a relationship may exist between DNA-PK activity and the defect responsible for Werner syndrome; it also suggests that DNA-PK may play a role in cellular senescence.

The quantitative peptide assay for DNA-PK permitted us to assay DNA-PK activity in cell cultures from a variety of sources. All normal human cell lines have similar levels of activatable DNA-PK. Two African green monkey cells lines also had levels of activatable DNA-PK that were similar to the levels in human cell extracts. Surprisingly, all rodent cell cultures examined had at least 20-fold less DNA-PK activity than primate cells. This result suggests that primates and lower animals may regulate DNA-PK expression or DNA-PK activation in different ways. Rodent cells also lacked the 350-kDa polypeptide that is detected with our anti-DNA-PK sera.

To confirm that the 350-kDa polypeptide is encoded by chromosome 8, human-mouse hybrid cells were obtained that contained only human chromosome 8 or human chromosome 7. Only the hybrid with human chromosome 8 expressed the 350-Kda polypeptide; however, no DNA-PK activity was observed in extracts of these cells even though the level of expression of the 350-kDa polypeptide was similar to the level in normal human cells. This result indicates that a second component (in addition to the 350-kDa polypeptide) is required for active DNA-PK.

A bacterial plasmid expression vector with a leader sequence containing six consecutive histidine residues was constructed, and the POU domain of the human octamer (Oct-1) transcription factor was subcloned into it. A two step affinity chromatography method for purifying the Oct-1/POU fusion protein was devised, and the purified fusion protein was shown to be a substrate for DNA-PK. Efforts to identify the amino acid residue(s) phosphorylated by DNA-PK are in progress.

An analysis of DNA-PK activation using human adenovirus DNA suggests that efficient activation in vitro requires physical breaks in the DNA or the availability of DNA ends. This finding suggests that in vivo DNA-PK may be activated by breaks in chromatin such as those produced by x-rays and ionizing radiation. DNA-PK was found to phosphorylate the 34-kDa subunit of replication protein A (RPA); in vivo, phosphorylation of RPA is strongly stimulated by ionizing radiation. RPA is a single-stranded DNA binding protein that is required for DNA replication and also functions in recombination. Thus, in addition to its putative role in transcription, DNA-PK may affect cellular DNA replication and recombination.

The findings presented above together with recent studies on the role of phosphorylation in regulating human p53 expression suggest a model where activation of DNA-PK by DNA damaging agents may stabilize p53 that is bound to chromatin. p53 is a cellular tumor suppressor gene that is altered or

deleted in most human tumors. Elevated levels of p53 block cell growth and may induce cell death. We suggest, therefore, that DNA-PK may function in this human tumor prevention pathway by activating the tumor-suppressing potential of p53 in response to specific signals that are produced when cellular DNA is damaged by ionizing radiation, chemicals, and other agents. Efforts to test this hypothesis will be pursued.

NOTE: This project does not involve human subjects. This project does not involve vertebrate animals.

PAPERS/JOURNALS/PUBLICATIONS:

1. Lees-Miller, S.P., and C. W. Anderson. The DNA-activated protein kinase, DNA-PK: A potential coordinator of nuclear events. Cancer Cells 3, 341-346 (1991).

2. Lees-Miller, S.P., K. Sakaguchi, S. Ullrich, E. Appella, and C.W. Anderson. The Human DNA-Activated Protein Kinase Phosphorylates Serine 15 and 37 in the Aminoterminal Transactivation Domain of Human p53. Molecular Cell. Biol. (in press) (1992).

3. Anderson, C.W. and Lees-Miller, S.P. The Human DNA-activated Protein Kinase, DNA-PK. Critical reviews in Eukaryotic Gene Expression 2, (in press) (1992).

FOLLOW-ON FUNDING:

1. NIH proposal re-approved for funding Feb. 1992: "The DNA-activated protein kinase: Cloning and mapping" (PIQ 91-1-1).

PROJECT NUMBER: 91-18

PROJECT TITLE: Development of an Ultra-Fast Laser System for Accelerator Applications

PRINCIPAL INVESTIGATOR: White, Mike

LDRD FUNDING:

FISCAL YEAR		AMOUNT
1990		$ 0
1991		79,657
1992		99,993
1993	(authorized)	0
1994	(estimated)	0
TOTAL		$179,650

PROJECT DESCRIPTION:

It is proposed to develop a prototype, ultrafast laser system capable of serving as a photocathode source for high-brightness electron guns and a "seed" laser for harmonic generation and amplification in a wiggler power-amplifier VUV source. The laser will be based on a regenerative amplifier scheme and will feature high repetition rate (>5kHz), complete tunability from 1000 nm to 300 nm, high peak energy (> 25μJ) and ultrashort pulses (6 psec). Initial experiments will be performed at the ATF facility on photocathode development and ground work for injection seeding into wiggler devices.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: The goal of this project is to develop a prototype laser system capable of serving both as a photocathode pump source for LINAC injection as well as a tunable seed laser for harmonic generation and final amplification a free electron laser operating in the vacuum ultraviolet (VUV-FEL). Such a device is currently under design consideration by the NSLS and laser development is crucial to the design and performance of the proposed facility.

APPROACH: The prototype photocathode/ seed laser system uses a regenerative amplification scheme to directly amplify weak, broadly tunable, picosecond pulses from a conventional, synchronously-pumped dye or Kerr-lens titanium sapphire mode-locked laser. High-repetition regenerative amplification schemes have been developed in the Chemistry Department for fixed IR frequency light and seed laser development will draw heavily on this experience. This approach should be capable of producing ultrafast pulses (6 psec) of radiation between 1000 nm to 330 nm with pulse energies greater than 25 μj. Our current regenerative laser system in the Chemistry Department can produce gigawatt peak powers at repetition rates up to 3 KHz and rates as high as 6 KHz should be possible. The utilization of all solid state components also ensures maximum reliability and low maintenance costs.

TECHNICAL PROGRESS FY 92: We have successfully completed the implementation of the titanium sapphire regenerative

amplifier and the high repetition rate oscillator consisting of a mode-locked Ti-Sapphire laser which can produce tunable radiation from 720 nm - 1100 nm with ultrafast pulse lengths approaching 100 femtoseconds. The production of such ultrafast laser pulses will allow the eventual VUV-FEL facility to investigate the nuclear motion of chemical and biological molecules in real time. The titanium sapphire amplifier system current output characteristics are as follows; 150μJ per pulse at 780 nm, 180 fs pulsed compressed at a one kilohertz repetition rate. This output is achieved with 2mJ of 527 nm, 300 ns pump light from a Q-switched kilohertz Nd:YLF laser and has a slope efficiency of 25%. The titanium sapphire regenerative amplifier output is TEM_{00} with $\pm$ 1% energy stability. The excellent performance of this system have confirmed our choice of Ti-Sapphire as the appropriate material for constructing a completely solid-state, tunable laser system in user applications where reliability is a major concern. Further characterization of the output is required with the anticipation of achieving short pulse durations ($<$ 100 fs) and a factor of five higher energies. This work will continue under the Basic Energy Sciences base program.

The final phase of the regenerative amplifier fabrication consists of characterization of the optical properties and production of 1 mJ output. The current limitation in energy is caused by the 2 mJ of pump energy available from the externally doubled Q-switched Nd:YLF laser. It is possible to produce five times more pump energy by intracavity doubling of the Q-switched laser which is now underway. In fact, intracavity doubled Q-switch lasers are now commercially available and should reduce the development burden for the FEL facility. This increase in pump energy will simply equate into an increase in the titanium sapphire regenerative amplifier output since the amplifier is not saturated.

PAPERS/JOURNALS/PUBLICATIONS:

1. "Proposed UV-FEL User Facility at BNL", I. Ben-Zvi, L.F. DiMauro, M.G..White and L.H. Yu, Nuclear Instruments and Methods, A304, 181-186 (1991).

NOTE: This project does not involve human subjects. This project does not involve vertebrate animals.

PROJECT NUMBER: 91-19

PROJECT TITLE: Cluster Impact Fusion

PRINCIPAL INVESTIGATOR: Friedman, L.

LDRD FUNDING:

FISCAL YEAR		AMOUNT
1990		$
1991		29,696
1992		49,766
1993	(authorized)	0
1994	(estimated)	0
TOTAL		$ 79,462

PROJECT DESCRIPTION:

Extremely high transient pressures and energy densities generated by accelerated cluster ion impacts have been shown to produce fusion of deuterons. Exploration of the fundamental aspects of the energy transfer processes in these cluster impacts is necessary to determine the utility of cluster impacts as a research tool and for the possible generation of fusion power.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: The specific purpose of this project is to investigate possible collective interactions of deuterons in cluster projectiles with target deuterons that might lead to enhanced rates of nuclear fusion reactions. Magnetic filtration of fully accelerated cluster ion beams has demonstrated that the unusually high rates of fusion observed in cluster impacts were largely the result of high velocity atomic or molecular deuterium artifact ions in the cluster beams. When these artifact ions were removed, by magnetic beam filtration, polydeuteroethylene targets were observed to show a rapid loss of sensitivity to cluster impact during the course of irradiation with the cluster beams. When these targets were removed, very thin conducting layers of carbon were observed on the target surfaces. The discovery that rates of target dehydrogenation were more rapid than overall sputtering rates and that very thin steady-state carbon films were generated by cluster impact provided a means of investigating rates of cluster fusion with very small cluster ions with a means of distinguishing between high- and low-velocity projectiles. These studies have provided preliminary evidence for small collective effects when heavy water trimers or tetramers are used as projectiles. Films of the order of 1000 A of carbon build up in times of the order of 1000s and reduce initial fusion rates by factors of 2-4. Careful determination of the widths of fusion proton energy distributions can be used to establish the energy of reactant deuterons. This eliminates the argument that contaminant monomer or dimer heavy ions are responsible for any enhanced rates of fusion observed. The magnitudes of enhancements for clusters containing five heavy-water molecules are roughly a factor of two over estimated thick-target yields.

The formation of thin carbon films on targets eliminates the possibility of a search for cluster fusion with projectiles containing

more than five or six water molecules accelerated to energies of less than 750 KeV. Solid heavy-water targets are currently under investigation to determine if problems with local pressure generated by beam sputtering can be overcome with the use of pulsed ion beams. These targets are "self renewing" and can be used for long-time studies of low yield reactions.

In addition to direct investigations of fusion reactions with cluster beams, an effort has been mounted to develop a multicharged ion source capable of delivering molecular ion beams with up to hundreds of charges with sufficient intensity to be useful in the investigation of fusion reactions. So far multicharged ion sources have been used mainly in analytical biochemistry. Considerable progress has been made in increasing the collection efficiency of ions from electro-spray sources. Techniques that have been developed earlier in our laboratory for ion post-acceleration and the determination of secondary electron yields have been used to directly characterize the charge states of ions produced in the electrospray process.

PAPERS/JOURNALS/PUBLICATIONS:

1. Fusion reactions in dense hot atom assemblies generated by cluster impact. R. J. Beuhler, G. Friedlander and L. Friedman, Acct. Chem. Res. 24, 198-202 (1991).

2. Cluster impact fusion. R. J. Beuhler, G. Friedlander and L. Friedman, Phys. Rev. Lett. 63, 1292-1295 (1989). Erratum: Phys. Rev. Lett. 68, 2108 (1992).

3. Deuteron-deuteron fusion by impact of heavy-water clusters on deuterated surfaces. R. J. Beuhler, Y. Y. Chu, G. Friedlander, L. Friedman and W. Kunnmann, J. Phys. Chem. 94, 7665-7671 (1990). Erratum: J. Phys. Chem. 96, 4724 (1992).

NOTE: This project does not involve human subjects. This project does not involve vertebrate animals.

PROJECT NUMBER: 91-21

PROJECT TITLE: Effect of a Bacterial Spore Protein on Mutagenesis

PRINCIPAL INVESTIGATOR: Setlow, J.K.

LDRD FUNDING:

FISCAL YEAR	AMOUNT
1990	$ 0
1991	87,461
1992	159,594
1993 (authorized)	162,000
1994 (estimated)	0
TOTAL	$409,055

PROJECT DESCRIPTION:

Mutagenesis will be studied in Escherichia coli with a cloned protein gene from Bacillus subtilis whose product drastically alters DNA configuration. In vitro mutagenesis will be investigated with pure protein product and pure Haemophilus influenzae transforming DNA.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: The purpose of this project is to investigate the molecular mechanisms of mutagenesis, a phenomenon believed to be responsible for most cancer in mammals. Our preliminary work has established for the first time that DNA configuration is a very important parameter in mutagenesis. The long-term objective is to find out how extra supercoiling of DNA leads to errors in DNA.

APPROACH:

(a) With the help of a mutant B. subtilis protein that fails to bind to DNA, to determine whether such binding is necessary for the mutagenic effect.

(b) To determine the effect of a number of different E. coli genes on this mutagenesis.

(c) To determine the single-base changes in E. coli caused by the Bacillus subtilis protein.

(d) To determine whether the mechanism of this mutagenesis involves the SOS system, in which a special error-prone DNA repair is induced by DNA damage. This may be done with lambda phage lysogens and with a recA-β-galactosidase gene fusion. Also the mutagenesis of the cloned gene itself caused by the B. subtilis protein may help the determination.

(e) Clone the B. subtilis gene into our recently constructed H. influenzae vector, and measure the mutagenesis in this bacterium lacking an SOS system.

(f) Measure in vitro mutagenesis with purified H. influenzae transforming DNA and B. subtilis protein.

TECHNICAL PROGRESS AND RESULTS:

(small letters refer to approaches, above)

(a) The B. subtilis protein has been shown to cause mutagenesis by binding to DNA, resulting in a configuration change of the DNA.

(b) The effect on such mutagenesis of five different mutations of E. coli that might be expected to affect the protein-caused mutations has been assayed.

(c) The single-base changes of 346 mutants made by the protein in E. coli have been determined.
d) The lambda phage lysogen showed little or no extra release of phage in the presence of large amounts of the B. subtilis protein. However, when the lysogenic cells were treated with ultraviolet (UV) radiation, there was about two orders of magnitude less phage release in the presence than absence of protein. Measurements of the protein produced by these cells confirmed that the protein inhibited the phage genes. Similarly, the protein greatly inhibited the B-galactosidase activity of UV-irradiated E. coli containing the recA-β-galactosidase fusion, a system which without the protein greatly increased the enzyme. The B. subtilis protein kills cells as well as mutagenizing them. The ability of the plasmid to accomplish these biological effects in E.coli was altered when the cells were allowed to replicate. The mutagenic property was lost after a small number of cell divisions, which had little effect on the killing ability of the plasmid, the latter being lost only very gradually. These three sets of data indicated that the SOS system is not responsible for the B. subtilis protein mutagenesis.

We have begun purifying and examining restriction enzyme patterns of plasmids carrying the B. subtilis gene intact and with altered biological properties. One plasmid with no ability to mutagenize or kill its host contained a sizeable deletion with the B. subtilis gene missing. Other plasmids which had lost mutagenic ability, but not killing ability did not contain obvious deletions. We are setting up to sequence these to compare them with the fully active plasmids.
(e) Initial attempts to grow in H. influenzae a hybrid plasmid containing the B. subtilis gene in our H. influenzae vector have failed. We believe that the copy number of the hybrid plasmid is too high, so that the cells cannot tolerate the amount of B. subtilis protein. We have now made a low-copy version of the cloning vector, and hope soon to succeed with this approach.
(f) H. influenzae transforming DNA was not mutagenized by binding to the B. subtilis is protein, probably because the protein was eliminated by cell protease before replication of the DNA occurred, if the protein entered the cell along with the transforming DNA. However, the complexed DNA had a large effect in rendering the transforming DNA resistant to UV radiation. The resistance of the complexed DNA was not affected by a mutation in H. influenzae host cells causing defective excision repair of DNA, a mutation which we showed earlier results in considerable added sensitivity of DNA without the protein. The post-replication repair system of H. influenzae did seem to operate on the UV-irradiated complexed DNA.

PAPERS/JOURNALS/PUBLICATIONS:

1. Mutation and killing of Escherichia coli expressing a cloned Bacillus subtilis gene whose product alters DNA conformation. Journal of Bacteriology 174: 2943-2950 (1992).

NOTE: This project does not involve human subjects. This project does not involve vertebrate animals.

PROJECT NUMBER: 91-23

PROJECT TITLE: Structure and Function of Adenovirus Penton Base Protein

PRINCIPAL INVESTIGATOR: Freimuth, Paul I.

LDRD FUNDING:

FISCAL YEAR	AMOUNT
1990	$ 0
1991	64,623
1992	138,877
1993 (authorized)	65,000
1994 (estimated)	0
TOTAL	$268,500

PROJECT DESCRIPTION:

The penton base protein of adenovirus is a protein of about 65,000 Da with multiple functions: it forms the 12 five-fold symmetric vertices of the icosahedral shell of the virus; it is the attachment site for fibers that bind specific receptors on the cell surface; and it is thought to be involved in penetration of the cell membrane, perhaps through an acid-induced conformational change in the penton base protein after endocytosis of the virus particle. The aim of this work is to understand these functions in terms of the structure of the penton base protein. A combination of physical, biochemical and genetic approaches will be used to characterize the multiple functions of the adenovirus penton base protein and to probe structure-function relationships.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: The purpose of this project is to investigate early stages during adenovirus infection, including the transport of virus particles across cell membranes, and the disassembly of virus particles, allowing delivery of the DNA genome to the cell nucleus. From the point of view of medical virology, adenovirus infection of immuno-suppressed individuals often is fatal, and therefore is life-threatening to organ transplant patients and to patients with diseases such as AIDS. Entry and uncoating are of considerable interest, since both stages in infections by other viruses can be blocked with drugs. Influenza virus, for example, can be blocked at the entry stage by compounds such as chloroquine that neutralize the pH of acidic organelles, or at the uncoating stage by amantadine, a small molecule that binds to virus and prevents an influx of protons that is necessary to disrupt virion stability. From the viewpoint of gene therapy technology, it is desirable to assemble virus-like agents in vitro from purified components. Adenovirus has potential to meet these goals, however further investigations are necessary to identify the cohesive interactions between virion components that drive assembly, and to understand how these bonds are broken during disassembly. When these aspects are understood, it may be possible to exploit the large DNA packaging capacity of adenovirus for gene transfer.

APPROACH: Our studies are focused on the adenovirus penton base subunit, which forms the 12 vertices of the icosahedral capsid. This protein is thought to function

both in entry and uncoating, based on earlier investigations. We have developed assays to assess functions of purified penton base, including its self association and pentamers, its assembly with another virion subunit (the fiber protein), and its interaction with cell adhesion molecules. Using site-directed mutagenesis of a cloned penton base gene, we are trying to identify domains that control these functions. In addition, the DNA sequences of penton base genes from distantly related adenoviruses are being determined and compared to the prototype to study sequence conservation. We expect that highly conserved regions will correspond to domains with functions that are shared by all adenovirus types, such as self-association or cohesive interaction with other capsid proteins, whereas nonconserved regions may correspond to domains with functions that are unique to a particular virus type, for example functions that may determine which tissues or cell types can be infected. Finally, we can evaluate the function of these domains in the context of virus reproduction by site-directed mutagenesis of the penton base gene in the adenovirus genome.

TECHNICAL PROGRESS AND RESULTS:
Only small quantities of penton base are produced during adenovirus infection of HeLa cells, so we constructed E. coli strains that over-express penton base. Although the protein is insoluble in E. coli, we found that it can be refolded to a conformation similar to the native protein. By electrophoretic analysis we showed that the refolded protein forms pentamers, and that these can assemble with purified adenovirus fiber subunits. These structures are currently being analyzed with the Brookhaven STEM to confirm that they are morphologically and physically similar to native capsid subunits.

This system makes possible the mutagenic analysis of pentamer formation and assembly with fiber. We observed that N-terminal fragments of penton base also form oligomers but fail to assemble with fiber. Monomeric full length protein also failed to associate with fiber. Alignment of amino acid sequences of penton base from three adenovirus serotypes showed highly conserved regions at both ends of the protein, separated by a central region of variable length and sequence. Taken together, these results suggest a two domain structure, and that the C-terminal domain may contain the fiber binding site. DNA sequence analysis of penton base genes from additional adenovirus serotypes is currently in progress and will provide important evidence supporting this structural model. We are attempting to obtain crystals of the full length protein, as well as N- and C-terminal fragments, for structural analysis.

Using our expression system, we determined the mechanism of a long-known activity of penton base - the so called "early cytopathic effect," characterized by rapid rounding and detachment of adherent cells upon exposure to the protein. We found a three amino acid motif, Arg-Gly-Asp (RGD), near the middle of the protein that is absolutely required for this activity. Cell adhesion molecules in the $\beta 1$ integrin family bind to RGD motifs in extracellular matrix proteins (e.g. fibronectic), causing cells to attach to the substratum. Soluble fragments of fibronectin that contain RGD motifs displace cells from the matrix, resulting in morphological changes that resemble the cytopathic effect of penton base. We showed that cells could adhere to plastic surfaces coated with the wild type penton base, but that mutant proteins lacking the RGD motif could not direct cell adhesion. Therefore, the cytopathic effect of penton base reflects its interaction with cell

adhesion molecules, resulting in detachment of cells from the culture surface. To study the significance of penton base-integrin binding during virus replication, we constructed four adenovirus mutants with amino acid substitutions in the RGD motif. All four mutants replicate in cultured cells with efficiencies similar to wild type adenovirus. Preliminary studies suggest that these mutations alter the pathway and kinetics of virus entry into the cytoplasm, and may effect capsid stability.

During our cell adhesion experiments we observed that the adenovirus hexon subunit also can bind to the cell surface under certain conditions. However, unlike penton base, hexon does not contain an RGD motif and does not cause rounding of adherent cells. This activity is interesting to us for two reasons; one, it may point to a novel cell adhesion molecule, and two, it suggests an alternative mechanism for virus entry--the interaction of hexon, the major surface protein of adenovirus, with the cell membrane.

PAPERS/JOURNALS/PUBLICATIONS:

1. The adenovirus type 2 penton base subunit binds to an Arg-Gly-Asp-directed cell adhesion molecule. To be submitted to J. Virology.

NOTE: This project does not involve human subjects. This project does not involve vertebrate animals.

PROJECT NUMBER: 92-04

PROJECT TITLE: High Resolution Fast X-Ray Detector

PRINCIPAL INVESTIGATOR: Rehak, P.

LDRD FUNDING:

FISCAL YEAR		AMOUNT
1990		$ 0
1991		0
1992		57,542
1993	(authorized)	0
1994	(estimated)	0
TOTAL		$ 57,542

PROJECT DESCRIPTION:
It is proposed to develop high resolution fast x-ray detectors. The collection of signal electron generated by interaction of x-rays with the silicon is based on a proven idea of silicon drift detectors. For the proposed application, however, the electrons will be transported to a small cylindrical anode which is at the same time the gate electrode of a transistor integrated on the detector. This configuration reduces the detector capacitance to the minimum and the analysis show that the noise level down to several electrons r.m.s. is attainable at the room temperature. This noise performance is obtained with rates of two orders of magnitude higher and detector areas of factor of ten larger than present x-ray detectors. A significant increase in detection sensitivity can be achieved in all fields using x-rays.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: To develop high resolution fast x-ray detectors is a one year project. The purpose of the work during FY 1992 is thus identical to the project description. The funds for the project were made available in February 1992.

APPROACH: To use the resources in a most effective way the smaller prototypes of the intended detector were designed. These prototypes were placed on the periphery of masks used to fabricate a large linear drift detector for the STAR project at RHIC. The transistors on the prototype are identical to the transistors intended for the final product. The cylindrical electrode structure of the electron transport region is also similar to the structure intended for the full size detector, but is substantially shorter and the area of the prototype detector is much smaller. However, the area is sufficiently large to test the transport properties of electrons in the cylindrical structures.

TECHNICAL PROGRESS AND RESULTS: The mentioned prototypes of the x-ray detectors were designed, produced and tested. There are four slightly different prototypes each having its positive features.

Fig. 1 shows the mask of both N and P implants on the N-side of one cylindrical detector magnified 14.3 times. The electrons produced by the conversion of x-rays within the silicon drift towards the center of the concentric rings where a collection anode and the first transistor of the read-out electronics are located.

Fig. 2 shows the anode region of the detector (center of the cylindrical structures of Fig. 1) magnified 550 times. All layers on the N-side are shown. Electrons are collected on the ring shaped anode which is connected to the gate of the first FET by an Al connection on the surface of the detector. The source of the transistor is a full circle in the middle and the drain is a ring between the anode and the gate. The transistor is a P-channel FET where the gate is realized by an N-implant and drain and source by p-implants.

Fig. 3 shows the measured characteristics, that is, the drain current I_D as a function of the drain to source voltage V_{DS} with the gate voltage V_G as a parameter. Please note the values of the gate voltages biasing the gate to channel junction in the forward direction. This bias is just opposite to the standard bias of the p-channel FETs, however, it is correct for our application where the leakage current of the detector is taking away through this forward bias junction.

There are problems with these characteristics. The drain current I_D is less than 1% of the expected value. It seems there is no conductive channel left under the gate. The root of it may be much deeper diffusion of the implanted atoms of phosphorus which form the n-gate. The simulation program does not treat the tails of the implant precisely enough. Perhaps the use of an arsenic implant to form the n-gate would have been more successful.

Fig. 4 shows an additional problem related to the transistor. It shows the drain current as a function of the voltage between the drain and the gate with the polarity corresponding to the gate to channel junction being biased in forward direction. The slope of the straight part of the curve is the conductance of the part of the channel between the gate and the drain contacts. The value of the conductance is about a factor of 10 smaller than the simulated value. We suspect the density of the positive charges in the Si-SiO_2 interface and the diffusion of phosphorus atoms from the gate to drop the value of the conductivity.

Before the design of the final detector, we will try transistors with different implants. We should mention here that the present problems are confined to the electronics and that the electrode transport structure works well as expected.

NOTE: This project does not involve human subjects. This project does not involve vertebrate animals.

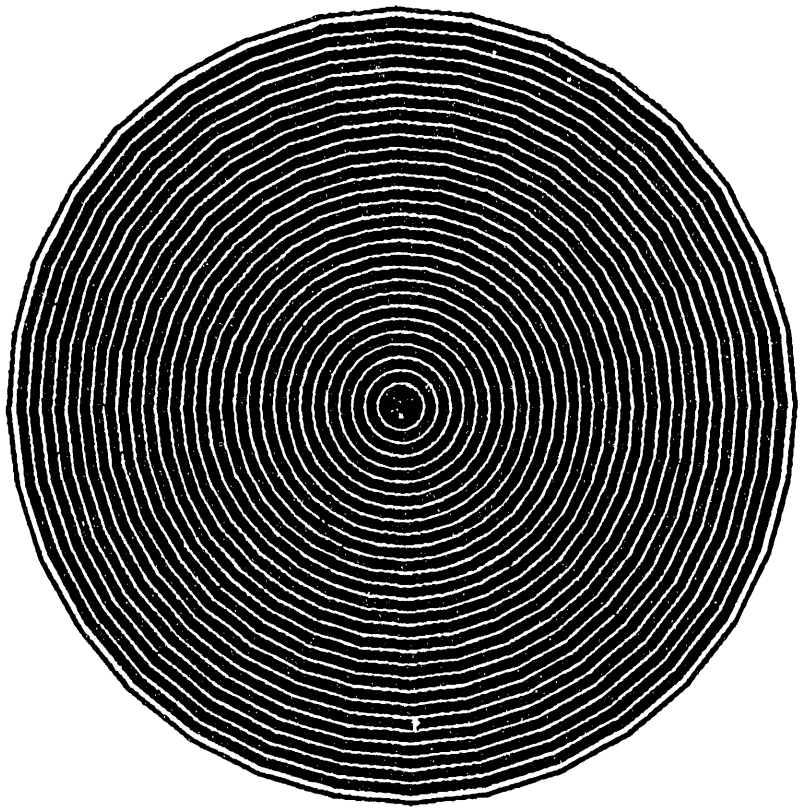


Figure 1 Layout of Cylindrical Detector.

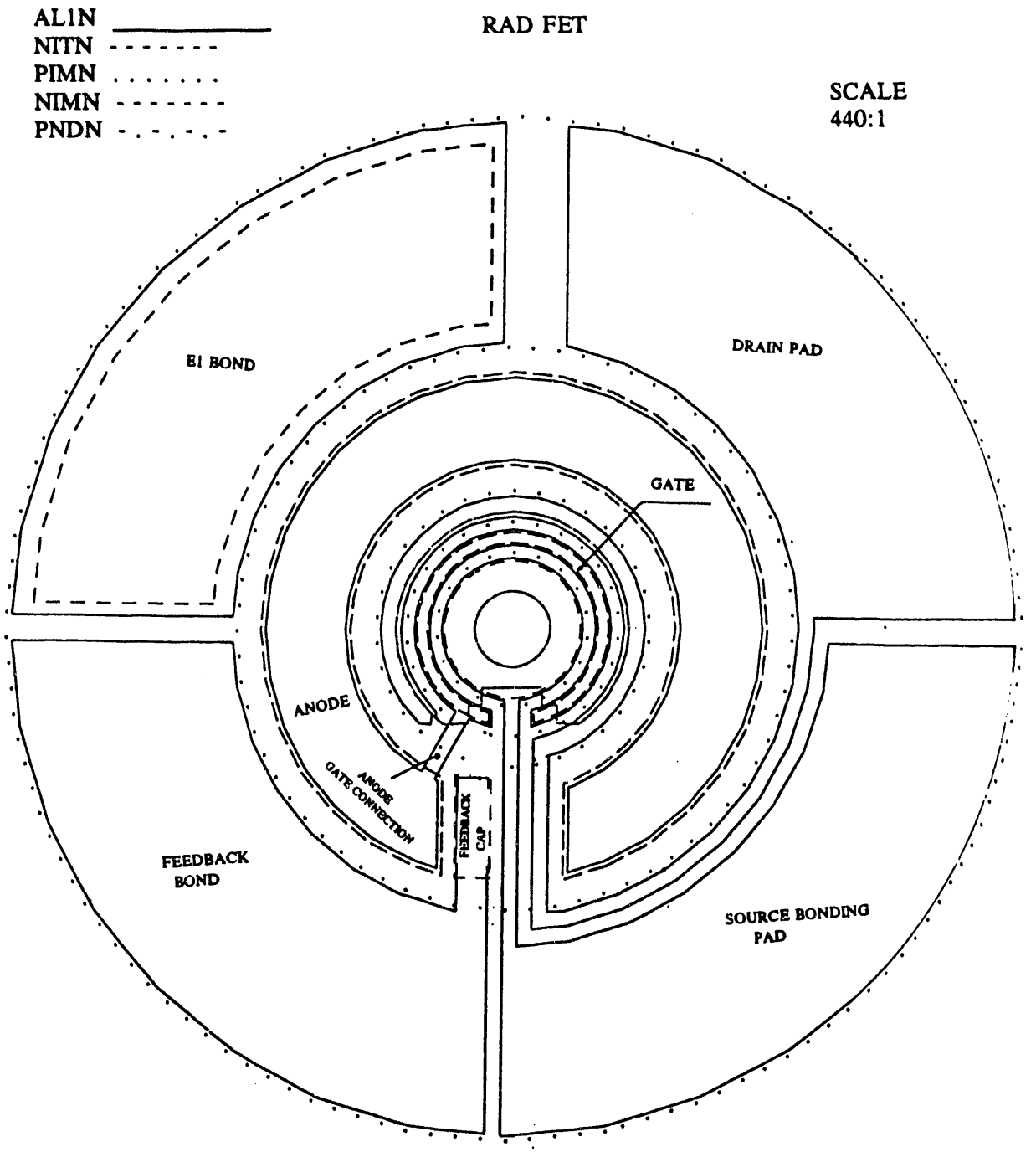


Figure 2 Integrated Electronics in the Center of the Cylindrical Detector.

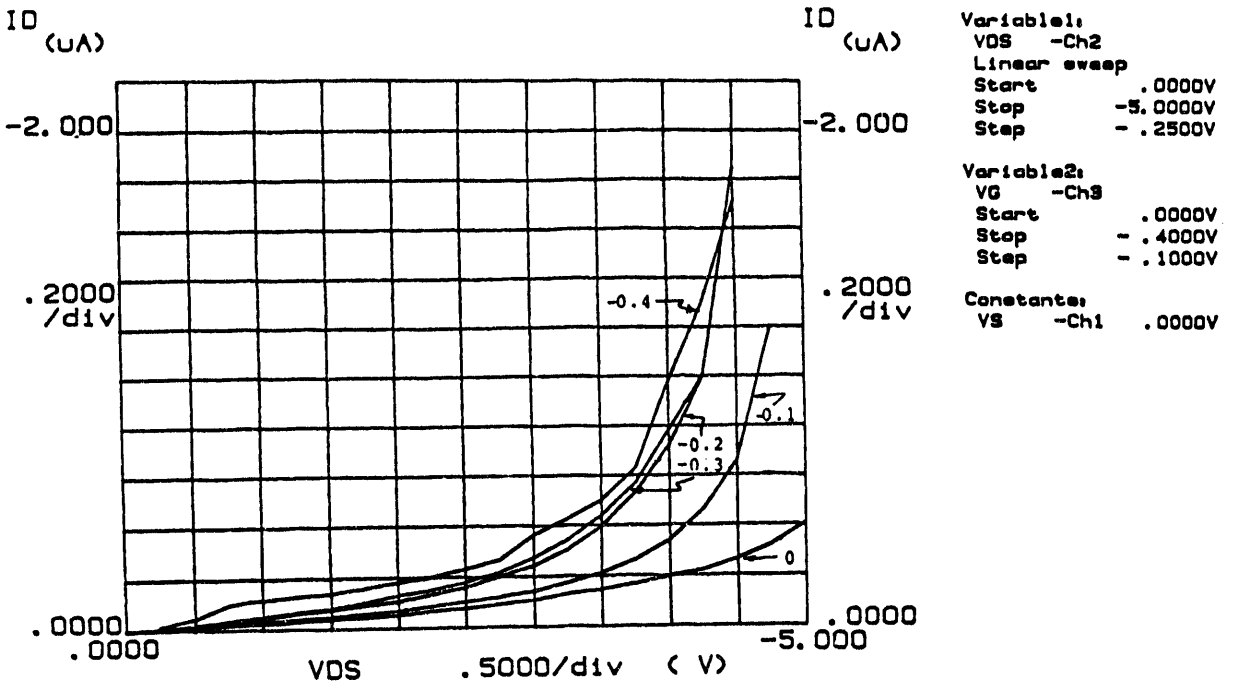


Figure 3 Characteristics of P-Channel FET.

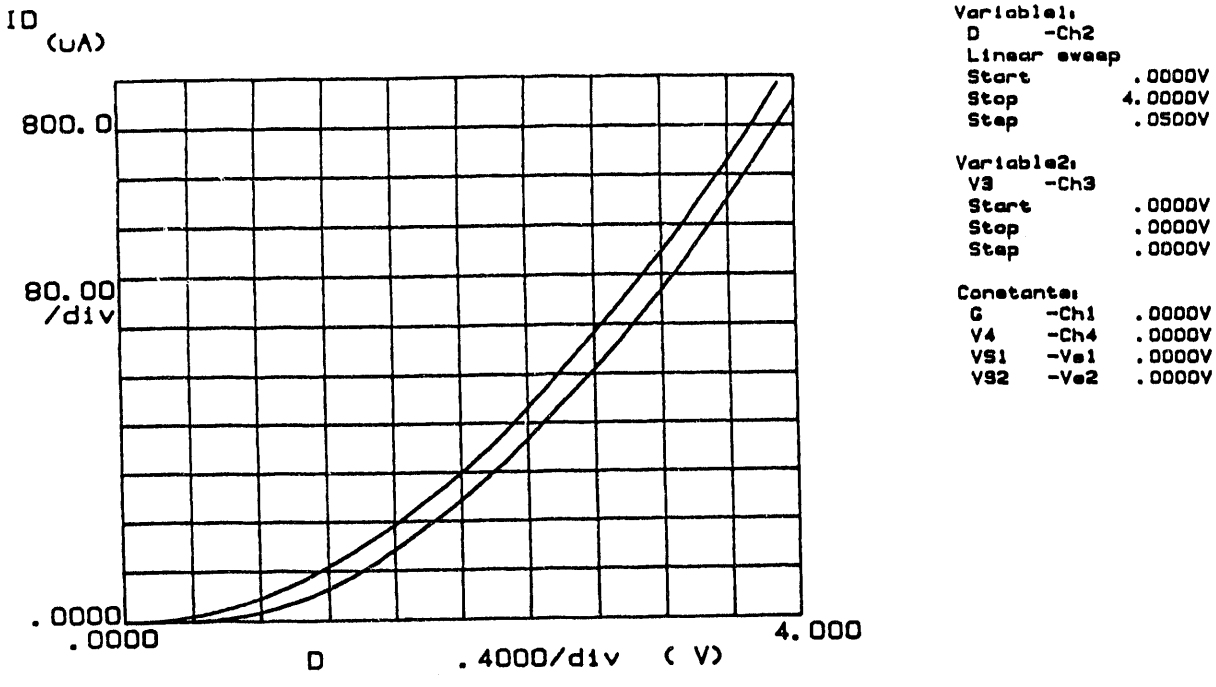


Figure 4 Measurements of the Conductivity of the Channel in the FET.

PROJECT NUMBER: 92-06

PROJECT TITLE: Coherent Synchrotron Radiation Longitudinal Bunch Shape Monitor

PRINCIPAL INVESTIGATOR: Blum, E.B.

LDRD FUNDING:

FISCAL YEAR	AMOUNT
1990	$ 0
1991	0
1992	40,000
1993 (authorized)	0
1994 (estimated)	0
TOTAL	$ 40,000

PROJECT DESCRIPTION:

It is proposed to monitor the shape of the electron bunches in the BNL Accelerator Test Facility (ATF) linac by analyzing the spectrum of the coherent synchrotron radiation emitted when the linac beam is deflected in the field of a dipole magnet. Because there is no fundamental limit in the time resolution that can be achieved with this technique, it will help in the eventual production of sub-picosecond bunches at ATF. Sub-picosecond bunches, in turn, will be a source of radiation with 100 micron to 1 mm wavelengths where tunable, high-power sources are not currently available. This may lead to the development of a new user facility at BNL.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: Radiation from a bunched electron beam undergoing an acceleration is emitted coherently at wavelengths that are comparable to the bunch length. The intensity of the coherent radiation is proportional to N^2, where N is the number of particles in the bunch, while the intensity of the usual incoherent synchrotron radiation is proportional to N. Since N can be greater than 10^9 in a bunch from a linear accelerator, an enormous enhancement in the spectrum is seen at long wavelengths. This enhancement has been seen in the spectra produced by a variety of processes: synchrotron radiation from electrons deflected in a magnetic field, transition radiation from electron passing through a metal surface, and Cherenkov radiation produced by passage through a gas.

According to theory and supported by previous experiments, the frequency distribution of the coherent radiation is proportional to the square of the Fourier transform of the longitudinal spatial distribution of the electrons in the bunch. Because the bunch length in a linear accelerator is on the order of 1 mm, the synchrotron radiation spectrum is coherently enhanced at mm wavelengths.

Frequency domain measurements of the mm wave spectrum will let us examine the electron bunch structure on a time scale that is shorter than can be measured by any existing time domain technique. Although a streak camera has been used for time

domain measurements of electron bunch shapes, they are difficult to use and, for the shortest bunches, are operating near their resolution limit. By contrast, the frequency domain measurements have no inherent resolution limit and will provide the means to study the shortest bunches that can be produced in a linear accelerator.

Production of sub-picosecond long electron bunches has been contemplated in several areas of accelerator physics including B meson factory and linear collider development. Frequency domain measurements will be helpful in examining these beams. The sub-picosecond bunches will, in turn, be a source of high power, continuously tunable, coherent radiation in the 100 micron to mm wavelength regime where no high power sources are currently available. This will be useful to experimenters in fields from biology to solid state physics and may lead to the creation of new user facilities.

APPROACH: Coherent mm-wave radiation will be used to measure the shape of the electron bunches in the BNL Accelerator Test Facility (ATF) linac. The frequency spectrum of the radiation emitted by the electron beam will be measured using a polarizing Michaelson interferometer. The Fourier transform of the spectrum should tell us not only the length of the bunch, but also the detailed shape including any substructure. The monitor will be capable of measuring both the synchrotron radiation produced in a bending magnet and the transition radiation from the electrons striking a copper target.

The electron bunch length in the ATF can be varied continuously from 1 psec to 9 psec by changing the duration of the laser pulse that is used to stimulate the photocathode of the electron gun. Different bunch lengths will be required for the different experiments that are planned for the ATF. Because dynamic effects can increase the length of the bunch during acceleration, it is important to measure the bunch length at the end of the linac. These results will be compared with time domain measurements for a new test of the theory of coherent synchrotron radiation.

If the initial bunch length measurements are successful, we will try to produce sub-picosecond bunches in the ATF linac. We may be able to accomplish this by magnetic compression in the linac injector or by exciting the photocathode with sub-picosecond laser pulses. The coherent radiation spectrum will be the only means available to study the results of these experiments.

TECHNICAL PROGRESS AND RESULTS: A scattering chamber with a z-cut crystal quartz window has been installed in the diagnostic beam line at the ATF linac to extract the coherent radiation. The chamber contains a rotatable copper mirror to direct the radiation onto the spectrometer. It can collect the synchrotron radiation from a bending magnet or, if the magnet in unpowered and the electron beam is allowed to strike the mirror, can be a source of back-scattered transition radiation. The first experiments to observe and measure the angular distribution of the radiation with a pyroelectric detector will be conducted when picosecond bunches are available from the ATF linac under our ongoing Basic Energy Sciences (BES) program.

The frequency spectrum of the radiation will be measured using a polarizing Michaelson interferometer which has been constructed using capital equipment funds provided by the National Synchrotron Light Source (NSLS). The detector will be tested in the

U4 IR beam line at NSLS and then moved to ATF. Bunch length measurements will begin in the Fall of 1992.

NOTE: This project does not involve human subjects. This project does not involve vertebrate animals.

PROJECT NUMBER: 92-07

PROJECT TITLE: High Gain Harmonic Generation Experiment

PRINCIPAL INVESTIGATOR: Ben-Zvi, I.

LDRD FUNDING:

FISCAL YEAR		AMOUNT
1990		$ 0
1991		0
1992		100,000
1993	(authorized)	100,000
1994	(estimated)	0
TOTAL		$200,000

PROJECT DESCRIPTION:

The purpose of this proposed research is to develop the technique of frequency multiplication in a Free-Electron Laser (FEL). The electron beam of the BNL Accelerator Test Facility will be used in a wiggler assembly to triple and amplify the seed radiation provided by a CO_2 laser. This technique is an essential element in the UV-FEL User's Facility being proposed by the NSLS Department. BNL will be in a better position to substantiate the technology required for such a facility.

A successful demonstration of the high gain harmonic generation principle will be an important step towards the realization of high power, tunable very short wavelength radiation sources. FELs based on this principle fall into the category of fourth generation synchrotron radiation sources and will play a significant role in photochemistry, atomic and surface physics, biology and other sciences. Therefore, this proposal supports the mission of the Basic Energy Science Division of the Department of Energy.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: The seeded single pass FEL has many advantages over other FEL concepts. The output bandwidth is controlled by the input seed, limited only by the pulse length, and a bandwidth of 10^{-4} is possible. Similarly, the frequency stability is also controlled by the seed hence the electron beam energy stability influences only the output intensity fluctuations, and the requirement on the energy stability is largely relaxed. Another obvious advantage is that the mirror loss and damage problems of FEL oscillators are eliminated. In addition, there is no need for a long train of micropulses. The electron beam can consist of single micropulses with the high repetition rate available from a superconducting linac. Thus, it is possible to achieve very good energy stability and high average power.

There are powerful, high repetition-rate tunable lasers operating in the IR and visible frequency bands that may be harmonic-multiplied into the VUV and used as seed lasers for the FEL amplifier. The interest in harmonic generation in FELs stems from the limitations of conventional laser harmonic generation techniques, such as low conversion efficiency, susceptibility to damage and limited tunability.

The generation of harmonics followed by exponential growth and wiggler tapering has been proposed and studied in detail as the basis for UV-FEL User's Facility at BNL. However, the complete process of generating the harmonics by prebunching in the fundamental and amplification in a wiggler tuned to the harmonic has not been demonstrated experimentally yet. The purpose of the High Gain Harmonic Generation Experiment is to pursue this study experimentally.

APPROACH: In the proposed harmonic generation experiment, we will demonstrate the bunching of a 30 MeV electron beam by a CO_2 laser of about 1 MW input power. We will study the super-radiant growth of the third harmonic at a wavelength of 3.47 microns, the exponential growth regime, and finally a tapered wiggler FEL amplifier section. We would like to verify our theoretical models and to answer important questions such as the effect of electron beam parameters on the coherence of the FEL, the effect of wiggler and alignment errors, and the higher harmonic contents of the FEL output as a function of the level of saturation.

The harmonic generation experiment is proposed for the BNL Accelerator Test Facility (ATF). We have selected the parameters of the harmonic generation experiment to match the electron beam parameters that have already been demonstrated experimentally at the ATF. These include a normalized rms emittance of 4 π mm mrad at a peak current of 130 amperes, an energy of 30 MeV, a CO_2 oscillator with a power of a few MW and solid-state optical chopping of the CO_2 laser to 10-100 picosecond long pulses.

We use an electromagnet wiggler constructed of many short sections that may be powered independently. The technology of a wiggler suitable for this purpose has been developed at the National Synchrotron Light Source at BNL under a previous LDRD 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.

TECHNICAL PROGRESS AND RESULTS: We have done a detailed magnetic design of the various wiggler sections. This included 2D simulations of the peak field; load curve and saturation behavior of the wiggler, 3D simulations of the parabolic pole faces and more. For the dispersion magnet, we have analyzed the magnetic design, determined the focussing properties, and considered the effect of departures from ideal design on the FEL gain. In the modulator and radiator wigglers, we have analyzed the effects due to the ends of the wiggler and developed methods for their correction. In addition, the localized field produced by a trim coil for horizontal beam steering has been investigated.

Then we have constructed a few wiggler sections with the parameters of the radiator wiggler of the High Gain Harmonic Generation Experiment, that is a wiggler period of 1.8 cm, a gap of 8 mm and parabolic pole face focussing. The length of each section is about 25 cm. To test these wiggler sections, we have developed a cryogenic wiggler magnet measurement system. The system scans the wiggler with a computer controlled Hall-Probe cluster in a liquid helium environment to determine the field quality.

In cryogenic test, we have determined that

the wiggler prototype sections performed to the peak field predicted by the load curve calculations and have begun a study of the wiggler errors. In particular, we have identified two error sources that have been introduced in the manufacturing and testing process and we are working on their reduction.

The beam line of the experiment has been designed, detailed measurements and improvements of the seed laser have been made, electron beam diagnostics and optical diagnostics have been designed and are under fabrication.

PAPERS/JOURNALS/PUBLICATIONS:

1. I. Ben-Zvi, A. Friedman, C.M. Hung, G. Ingold, S. Krinsky, L.H. Yu, I. Lehrman and D. Weissenburger, Design of a Harmonic Generation FEL Experiment at BNL. Nucl. Instr. & Meth. in Physics Res. A318, (1992).

2. X. Zhang, I. Ben-Zvi, G. Ingold, S. Krinsky and L.H. Yu, Analysis of the Superconducting Wiggler Magnets for the ATF Harmonic Generation Experiment. To be published.

NOTE: This project does not involve human subjects. This project does not involve vertebrate animals.

PROJECT NUMBER: 92-09

PROJECT TITLE: BNL Maglev Studies

PRINCIPAL INVESTIGATOR: Wegrzyn, J.

LDRD FUNDING:

FISCAL YEAR		AMOUNT
1990		$ 0
1991		0
1992		59,548
1993	(authorized)	40,000
1994	(estimated)	0
TOTAL		$ 99,548

PROJECT DESCRIPTION:

BNL is a leader in superconducting magnet development for high energy physics. The objective of this proposal is to transfer this expertise to maglev technology. Maglev is a high speed ground transportation system that uses superconducting magnets to levitate, guide and propel vehicles over elevated guideways at speeds of 300 miles per hour. The intent of this work is to place BNL in a position to receive from the National Maglev Initiative (NMI) funds for developing prototype superconducting magnets for maglev. Congress recently passed a $750 million appropriation in support of NMI to develop a US-Maglev system. The National Maglev Initiative is composed of the Department of Transportation, the Department of Energy and the Army Corps of Engineers. The result of this work will be a comprehensive proposal to NMI that establishes BNL as an appropriate site for designing and testing prototype magnets for maglev. Tasks to be performed under this Exploratory Research Proposal are data gathering (Task 1), computer simulation studies (Task 2) and testing procedures (Task 3) for developing superconducting maglev magnets.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: The purpose of this project is to identify directed research opportunities for Brookhaven within the emerging National Maglev Program. Brookhaven has a long history associated with maglev starting with its concept by BNL scientists Dr. Jim Powell and Dr. Gordon Danby. Since BNL is a leader in the key technology of superconducting magnet development, it has a natural role to play in maglev. This project looks at linking the needs of the US maglev program to the demonstrated technical capabilities of BNL.

APPROACH: The approach is to build upon BNL expertise in superconducting magnet design by presenting to the National Maglev Program a comprehensive work plan on developing and testing prototype maglev magnets. To have the proposal favorably reviewed it is necessary to show that funding R&D at Brookhaven will reduce the government's overall cost and risk associated with maglev development. To this end the two problems of magnet fatigue and sequential magnet quench have been identified as meeting this reducing cost/risk criteria.

Magnet fatigue is the gradual degradation of magnet performance with many hours of service, leading possibly to a quench of the superconducting magnet. Since full scale, test track measurement of magnet fatigue for determination of mean-time-between-failure is time consuming and expensive, laboratory testing of magnet fatigue can reduce both cost and risk associated with maglev development. Needless to say, an unscheduled quench of a magnet sub assembly (bogie) during test track runs is not desirable. Even though maglev vehicles can be designed with redundant magnet lift capability, the quench of one magnet in a bogie can trigger the quench of the adjacent magnets due to their inherent magnetic and thermal coupling. Laboratory tests to guard against sequential magnet quenching is another area of directed research which can again reduce government's cost and risk factors.

The following four tasks are being developed for submission to the government's Maglev Task Force for future funding:

I) Magnetwire Studies
II) Reference Modeling and Stray Field Effects
III) Magnet Fatigue Problem
IV) Sequential Magnet Quench

The chronological approach is to start with the generic research topics such as tasks I and II, and as the maglev program matures move into tasks III and IV. Tasks III and IV require the use of a magnet test stand. The test stand consists of suspending superconducting magnets over an array of stationary but time dependent current sources that simulate in field strength the conditions of magnets moving across a passive guideway. Since this work is at its mid-point, the following discussions are a general overview of the technical progress to date.

TECHNICAL PROGRESS AND RESULTS: Technical progress has been realized in the two areas of developing a 3-D Magnetic/Guideway Reference Model and in the designing and costing of a BNL Maglev Test Stand for magnet testing.

A 3-D MAGNETIC/GUIDEWAY REFERENCE MODEL

Modeling/EM Fields: One rationale for the work is with the need for reference models when evaluating stray EMFs, propulsion, guidance, levitation, and AC loss mechanisms for maglev. The work can be classified as further developing government in-house assessment capabilities for direct comparisons of the relative strengths and weaknesses of the four commercial System Concept Definition (SCD) designs. The work is an outgrowth of ongoing activities at BNL, so these results can be a factor in the government's next screening process in selecting industrial contractors.

Past studies, most notably GE BAA-35, indicated the difficulties in reducing the magnetic fields in the cabin area to below 5 Gauss(dc). This conclusion was based on proprietary GE software. The SCD contractors, on the other hand, report that 5 Gauss fields are readily achievable with their designs. These statements are based on using either 2-D or 3-D analysis with either proprietary or commercial software. A verifiable and reference method of determining 3-D stray fields is needed in order to make a fair comparison between competing designs. This is one objective of the work.

Stray field shielding decisions influence directly propulsion, levitation and guidance schemes. That is to say, changes to meet

stray field requirements may adversely impact vehicle performance. A generic computer model has been developed at BNL that analyzes the induced guideway currents caused by moving magnets. (See Guideway Induced Current.) Parametric studies are possible using this model that predict the effects of magnetic/guideway design changes. Benefits from this study are verification of SCD performance claims.

Magnetic fatigue, resulting in a superconducting quench, is a major concern. Magnetic fatigue is associated with the long term exposure to both mechanical and electrical vibrations. BNL from its low T_c AC Transmission Line Project has unique expertise in designing against magnetic fatigue. Under this task design options that mitigate the problem of magnetic fatigue will be identified. The findings will be made available to the maglev magnetic design community under the auspices of technology transfer.

Guideway Induced Current: If a magnet (first circuit) is moving with a constant velocity over a conductor (second circuit), an induced current will occur in the conductor. For discrete coil geometry, this induced current can be calculated by solving a first order ordinary differential equation involving resistance, self-inductance, current in the second circuit, and the mutual inductance. The induced current i_2 in a secondary (track) loop is governed by the equation

$$L\frac{di_2(t,x)}{dt}+Ri_2(t,x) -\Sigma M_j(i_2(t,x_0+jl) +i_2(t,x_0-jl))+\frac{d\phi}{dt}=0$$

where L and R are the self-inductance and resistance respectively. Greek letter phi is the magnetic flux and is equal to the product of a constant current in the main (train) loop, and the mutual inductance M. The terms involving the summation sign represent the electromagnetic interaction from all the other track loops. M_j is the mutual inductance between the train loop and the jth track loop. 1 is the center-to-center distance between the track loops.

Assuming constant velocity v for the track loop, the location for the center of the track loop at time t is represented by $x_0 + vt$ where x_0 is the initial location. Substitute this into the expression for M, the magnetic flux is now time-dependent. Furthermore, the induced current and magnetic flux are also assumed to be zero at $t = 0$. While the above equation together with the assumed initial conditions can be solved numerically, it can be solved just as easily by the Laplace Transform method to yield an analytical solution.

This is the basis for the BNL Maglev Test Stand in which the condition of a moving magnet over a passive guideway is replaced by a fixed magnet over an active guideway.

BNL MAGLEV TEST STAND

Testing of superconducting prototype magnets for maglev applications requires the development and construction of active guideway coils. In a functioning maglev system the guideway coils are excited by the passage (transit) of the superconducting magnets, housed in the vehicle. The coils are passive and dissipate power only during the interval of time that the vehicle is above the coil; generally self-heating is of concern but not a problem. The proposed Maglev Test Stand simulates an active guideway, requiring an exciting power supply. The coils will be excited for long intervals of

time such that self-heating is a problem and the facility requires a cooling system.

A case study approach was taken by designing an active guideway coil based on a typical maglev system described in the literature. The vehicle is 20 x 4 meters and has a weight of 40 tons. The vehicle is levitated by 4 magnets, bogie assemblies. Each magnet is excited by 2000 kilo-ampere turns and each bogie has a lift of 10 tons. The guideway coils are 3.25 x 0.5 meters. Depending on the pitch, three guideway coils interact with an individual vehicle magnet. The magnetic lift forces experience a $\pm$ 10% on pulse at a rate that is dependent on the vehicle velocity and guideway pitch, and can be as high as 40 hz. The pulsations generate mechanical oscillations of the vehicle and its effect on the performance and stability of the superconducting magnets can be studied.

The cooling system is an integral component of the electrical design. Initially we considered the coils immersed in a temperature bath. Three cases were developed.

Case 1: Commercial copper wire immersed in a water bath at 100°C utilizing the heat of vaporation for cooling.

Case 2: A high-conductivity aluminum alloy (1350) wire immersed in a liquid nitrogen bath at 77.2°K.

Case 3: Commercial copper wire immersed in a liquid nitrogen bath at 77.2°K.

Conclusions are that for the magnet fatigue problem, the preferred approach is to go with Case 1 the high first cost, but low operating cost water cooled copper system. Work is on-going to finalize the design of the test stand.

NOTE: This project does not involve human subjects. This project does not involve vertebrate animals.

PROJECT NUMBER: 92-13

PROJECT TITLE: Structural Investigations of Pt-Based Catalysts

PRINCIPAL INVESTIGATOR: Pandya, K.I.

LDRD FUNDING:

FISCAL YEAR		AMOUNT
1990		$ 0
1991		0
1992		68,334
1993	(authorized)	73,000
1994	(estimated)	0
TOTAL		$141,334

PROJECT DESCRIPTION:

Catalysis presents a significant opportunity for us to develop a new long range program that would take advantage of the unique capabilities of BNL (NSLS and HFBR). It is proposed to investigated the structure and electronic states of several Pt based monometallic and bimetallic catalysts using XAFS, ^{1}H and ^{129}Xe-NMR and HREM techniques. These investigations will provide fundamental understanding of the structure and activity of these catalysts. The Pt-based catalysts that will be investigated present legitimate scientific questions on their own (e.g. size of metallic clusters; interaction with the support; kinetics and mechanism of alloying of the bimetallic system such as Pt-Au; and quantification and reconciliation of results obtained by traditional means of assessing metal dispersion). At the same time, these systems are an entry into the important area of metal clusters supported on zeolites and other large surface area materials, around which it is hope to build a catalysis program.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: Heterogeneous catalysts consisting of small metal particles supported on high surface area materials have a key role in the industrial refining processes. Their catalytic properties strongly depend upon the local structure, shape and size of the metal particles and the location of the metal particles on the support. Although considerable research is carried out to develop and characterize new catalysts, a fundamental understanding of the "Synthesis-Structure-Performance" relationship is still lacking.

This research program is directed toward structural and electronic characterization of Pt-based monometallic and bimetallic catalysts. This information will be used to understand the effects of catalyst structure and composition on the catalytic properties.

Our initial study concentrates on various Pt based monometallic and bimetallic catalysts. These materials offer many interesting problems in the field of small metal clusters supported on zeolites and other large surface area materials, around which we hope to build a catalysis program. The future research efforts will be directed toward studying various models as well as industrial grade catalysts.

APPROACH: Structural characterization of the supported metal catalysts has always been a challenging problem because the metal particles are small (< 1 nm), nonuniform (in size and shape) and dilute (< 1 wt % metal loading). As a result, traditional diffraction techniques provide little structural information. The Extended x-ray Absorption Fine Structure (EXAFS) technique has been widely used to determine the local structure and to probe the metal-support interface for highly dispersed catalysts. The quantitative information about the particle size (distribution) and metal dispersion can be obtained by a variety of techniques including High Resolution Electron Microscopy (HREM), Nuclear Magnetic Resonance (NMR), Chemisorption and EXAFS. Each of these techniques has some advantages and limitations, and it is necessary to use two or more techniques to study the supported small metal particles. The electronic states (oxidation state and fractional occupancy in the metal d-band) is determined from the X-ray Absorption Near Edge Spectroscopy (XANES).

We are using in-situ EXAFS and XANES spectroscopies to determine the local structure, electronic states and size of the metal particles. The size of the metal particles is also determined from HREM, Xe-NMR and chemisorption measurements. The results obtained from these techniques are combined to obtain the size distribution function. The main advantage of EXAFS and XANES over other techniques is that the experiments can be carried out in-situ.

TECHNICAL PROGRESS AND RESULTS:

(i) Pt/NaY System

We have studied the local structure and metal particle size for a series of Pt/NaY catalysts at commercially relevant low metal concentration using in-situ EXAFS spectroscopy. A series of Pt/NaY catalysts were prepared by incipient wet impregnation of NaY zeolite followed by reduction at 300, 500 and 650 C respectively. The EXAFS results show that the average size of the metal particles increases as the reduction temperature increases. The average metal particle size for the sample reduced at 300 C is 12 A. Such particles can be accommodated inside the zeolite supercages. For the samples reduced at higher temperatures, the metal particles are too large to fit inside the supercages unless there is a framework collapse. Other research groups have shown that the average metal particle size depends upon the calcination temperature. However, a comparison of our EXAFS study with other studies (Pt/NaY catalysts prepared by ion-exchange technique and treated under various conditions) clearly shows that the average metal particle size is about 12 -13 A for reduction temperatures up to 360 C and it increases drastically at higher temperatures irrespective of the method of preparation and calcination temperature.

(ii) Pt-Au/Al_2O_3 System

EXAFS measurements were carried out at Pt-$L_{2,3}$ and Au-$L_{2,3}$ edges on a series of Pt-Au/Al_2O_3 samples with varying gold concentrations. The preliminary results show that the metal particles are smallest for the $Pt_{60}Au_{40}$ sample. Detailed EXAFS and XANES analysis is in progress.

PAPERS/JOURNALS/PUBLICATIONS:

1. EXAFS Investigations of Metal Particle Size for Pt/NaY Catalysts at Industrially Relevant Low Concentrations. To be submitted for

publication in the Journal of Catalysis.

NOTE: This project does not involve human subjects. This project does not involve vertebrate animals.

PROJECT NUMBER: 92-15

PROJECT TITLE: Studies on the Cellular Toxicity of Cocaine and Cocaethylene

PRINCIPAL INVESTIGATOR: Volkow, N.

LDRD FUNDING:

FISCAL YEAR		AMOUNT
1990		$ 0
1991		0
1992		31,837
1993	(authorized)	45,000
1994	(estimated)	0
TOTAL		$ 76,837

PROJECT DESCRIPTION:

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 the local anesthetic actions of cocaine lead to changes in cellular ion concentration through interaction with Na^+ and K^+ channels, it is planned 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. It is proposed, however, that the increased toxicity is brought about by the differential interference with ion exchange across membranes by alcohol and cocaine. This project will measure the effects of cocaine, cocaethylene, alcohol, and alcohol with cocaine on elemental distribution in the cell of the leech ganglia. It is hypothesized 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^{++}.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: As mentioned earlier, 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. 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. However, we did observe a dose dependent effect of cocaine on glial Na but not on neuron Na. 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. We have not yet completed the analysis of the cocaethylene effects; approximately half of our samples have been analyzed so far. Water content and elemental content of leech neurons and glial cells, with the exception of Na and Cl (neuron) was not affected by cocaine. These data suggest that cocaine may have a specific pharmacological affect on glial cells. 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. We anticipate completing the analysis of the cocaethylene dose response samples within a few weeks, and plan to determine what additional and further studies are indicated. We anticipate developing a formal NIH proposal upon completion of these initial pilot studies.

NOTE: This project does not involve human subjects. This project does not involve vertebrate animals.

PROJECT NUMBER: 92-17

PROJECT TITLE: Human Melanocyte Transformation

PRINCIPAL INVESTIGATOR: Sutherland, B.M.

LDRD FUNDING:

FISCAL YEAR		AMOUNT
1990		$ 0
1991		0
1992		81,463
1993	(authorized)	91,000
1994	(estimated)	0
TOTAL		$172,463

PROJECT DESCRIPTION:

Malignant melanoma is an invasive, often lethal cancer of human skin. In spite of its increasing incidence and devastating human consequence, the critical DNA alteration(s) in melanoma are unknown. We have devised a scheme for a transformation assay for human melanocytes which will identify partial and fully oncogenic transformants and their gene alterations. It is proposed to develop this system, use it to follow transformation of normal human melanocytes, and to isolate transforming gene(s) from human melanomas.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: To develop cellular and molecular methods for studying the response of human primary melanocytes to radiation. Although malignant melanoma is a disease of severe human consequence, the responses to UV of human primary melanocytes are poorly understood, in large part a consequence of their limited growth in culture, difficulty of <u>in vitro</u> growth, poor colony-forming ability, and complications of molecular measurements due to their pigmentation. We plan to work out solutions to these problems, allowing assessment of the responses of primary human melanocytes to UV at the molecular and cellular levels.

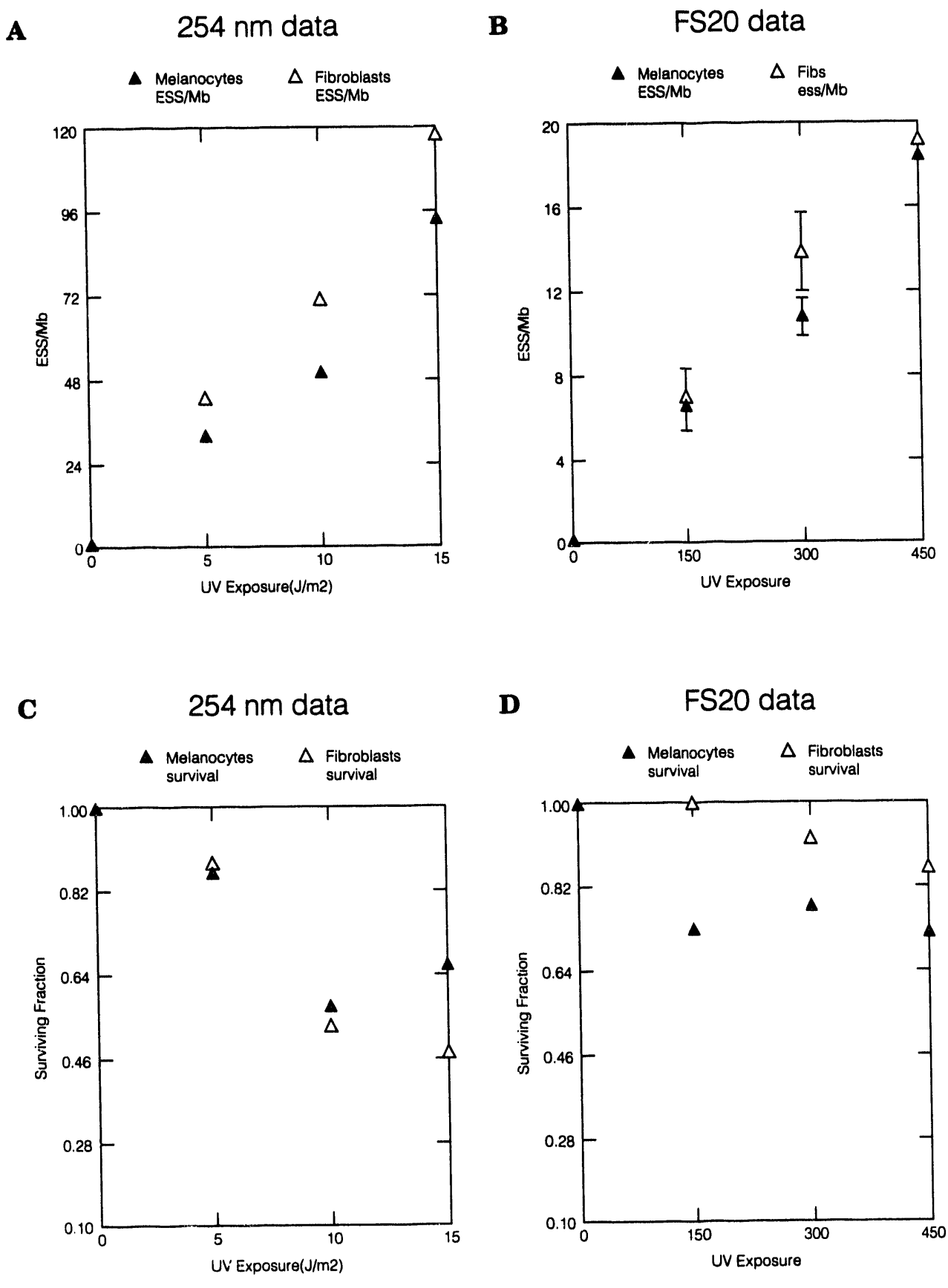
APPROACH: Primary cultures of human cells, including fibroblasts, keratinocytes and melanocytes are initiated from the foreskins of healthy neonates. Cells are exposed to UV of different wavelength regions [254 nm, broad spectrum UVB (290 -320 nm), or broad spectrum UVA (320-400 nm)], and the levels of pyrimidine dimers determined by the alkaline agarose gel method developed in the laboratory using the electronic imaging system of J. Sutherland; in addition, survival of cells under non-growth conditions (to simulate the situation in human skin) is determined by a new method we have developed using electronic imaging.

TECHNICAL PROGRESS AND RESULTS: Primary human melanocytes grow only to a limited extent in culture, form colonies poorly, and are available in limited numbers. Since colony-forming assays are precluded, methods of following cell survival after UV involve, e.g., harvesting

dishes of cells on sequential days by trypsin treatment, treatment with vital dyes, and determining the numbers of cells present. This method consumes large numbers of cells, which is both expensive and severely limits the experiments possible with primary cells. We therefore devised a non-destructive method which allows cell quantitation with minimum cell loss or disturbance: Cells are UV irradiated as usual, incubated for standard times, and the cell density determined periodically as follows: cells are kept warm while electronic images of several fields of each flask are recorded. The cells are returned to the incubator, and the images can be counted later at leisure without disturbing the cells. In addition, each culture can be counted on successive days, and the same culture can be used for transformation studies. At present the cell counts of the image are determined manually, but we are constructing a system which will allow computer-determined (investigator-edited) cell quantitation.

Fibroblasts have been widely studied, and provide a standard of comparison for melanocytes. We first determined the production of pyrimidine dimers (ESS) by 254 nm radiation in fibroblasts and melanocytes of the same donor; Panel A shows that 254 nm UV produced fewer dimers in the melanocytes (▲) than in the companion fibroblasts (△); in spite of the lower initial dimer frequencies, preliminary survival data indicate that the melanocytes survive only marginally better than the fibroblasts (Panel C). The FS20 (UVB) lamp produced similar frequencies in both cell types (Panel B), but the melanocytes seem to show more damaging effects of the radiation (Panel D). We are now obtaining independent replicates of these experiments, to determine whether these trends are general. These studies will provide the basis for determining the frequencies of transformation of melanocytes to independence from individual growth factors, and to possible oncogenicity by the UV wavelengths in sunlight.

NOTE: This project does involve human subjects. This project does involve vertebrate animals.


A
254 nm data
Melanocytes ESS/Mb
Fibroblasts ESS/Mb
ESS/Mb
UV Exposure(J/m2)
B
FS20 data
Melanocytes ESS/Mb
Fibs ess/Mb
ESS/Mb
UV Exposure
C
254 nm data
Melanocytes survival
Fibroblasts survival
Surviving Fraction
UV Exposure(J/m2)
D
FS20 data
Melanocytes survival
Fibroblasts survival
Surviving Fraction
UV Exposure

PROJECT NUMBER: 92-19

PROJECT TITLE: Exploratory Applications of X-Ray Microscopy; Determination of the Higher Ordered Structure of Eukaryotic Chromosomes

PRINCIPAL INVESTIGATOR: Van't Hof, J.

LDRD FUNDING:

FISCAL YEAR		AMOUNT
1990		$ 0
1991		0
1992		74,783
1993	(authorized)	80,000
1994	(estimated)	0
TOTAL		$154,783

PROJECT DESCRIPTION:

The x-ray scanning microscope at the NSLS provides an opportunity to determine whether or not such an instrument is capable of providing information about the higher ordered arrangement of chromatin in mitotic chromosomes. The range of resolution of the x-ray microscope is between that obtained by neutron diffraction and electron microscopy. Consequently, data from x-ray microscopy may be the bridge that connects findings obtained by neutron diffraction to those discovered by electron microscopy. The proposed work addresses three points:
(i) fine-tuning the hardware of the microscope to achieve maximum resolution of a metaphase chromosome in either a dry or wet state,
(ii) how to prepare chromosomes to maximize exploitation of the unique features of the microscope, and
(iii) determination of the folding patterns of the sub-chromatid fibers in three morphologically distinct sections of the chromosome, the kinetochore, the secondary constriction, and the telomere.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: The specific purpose of work undertaken during FY-1992 was:
(i) to evaluate radiation damage to chromosomes while being viewed with x-rays,
(ii) to determine the difference in the amount of damage between chromosomes viewed in the wet vs. dry state,
(iii) to determine the effect of fixation on chromosome damage and
(iv) to exploit the spectroscopic differences of DNA and protein so their distribution along the chromosome axis can be measured.

APPROACH: The overall strategy for the project falls into three categories: sample preparation, characterization of radiation damage, and establishment of new physical and chemical characteristics of chromosomes. Sample preparation encompasses development of a methodology that provides isolated undistorted chromosomes in quantity, on demand, with a minimum of work. This work will be

ongoing as there is always room for improving the quality of the samples. To view chromosomes with x-rays is to cause damage to them. Consequently, it is important to have an assay for damage so it can be minimized and its contribution considered when evaluating results. Finally, two characteristics of chromosomes are being pursued. The first is a test of the hypothesis that the mass fraction of DNA in eudaryotic chromosomes is a constant independent of size and source. The second, is to determine in thin-sectioned chromosomes the two-dimensional relation between coiled DNA and the protein attached to it.

TECHNICAL PROGRESS AND RESULTS: Radiation damage to Vicia faba chromosome structure, defined as mass loss, was determined in the Scanning Transmission X-ray Microscope (STXM) for unstained specimens in both the wet and dry states. Dried specimens remain undamaged after either single or multiple images at doses up to 2400 Mrad at wavelengths of 3.15 or 3.64 nm. In contrast, wet specimens are damaged irrespective of the imaging protocol. The damage induced by multiple exposures is greater than that seen in a single exposure of the same total dose. Thus, the rate of data collection is greater than or equal to the rate of damage. The damage during multiple exposures of wet chromosomes is influenced by several factors. First, the fixative used influences the extent of radiation damage. Wet chromosomes fixed with glutaraldehyde are more resistant than those fixed with formaldehyde or osmium tetroxide. A second factor is ionic strength. Damage to wet chromosomes increases if the ionic strength decreases below that at which chromatin undergoes a conformational transition. The mass of wet and dry chromosomes is the same, consequently quantitative measurements can be made on wet specimens. Such measurements give a DNA mass fraction of 39 +/- 8% for Vicia faba chromosomes.

PAPERS/JOURNALS/PUBLICATIONS:

1. Williams, S., Zhang, X., Jacobsen, C., Kirz, J., Lindaas, S., Van't Hof, J., and Lamm, S.S., Measurements of Wet Metaphase Chromosomes in the Scanning Transmission X-Ray Microscope. J. of Microscopy (in press).

NOTE: This project does not involve human subjects. This project does not involve vertebrate animals.

PROJECT NUMBER: 92-20

PROJECT TITLE: Uranium Neutron Capture Therapy (UNCT)

PRINCIPAL INVESTIGATOR: Hainfeld, J.F.

LDRD FUNDING:

FISCAL YEAR		AMOUNT
1990		$
1991		
1992		97,021
1993	(authorized)	104,000
1994	(estimated)	0
TOTAL		$201,021

PROJECT DESCRIPTION:

Uranium-235 appears to have advantages over boron-10 in neutron capture therapy. Its fission products have a greater range in tissue, 20-30 μm vs. 6-10 μm. On the average, a ^{235}U atom in the target yields 5- to 10-fold more ionization energy than does a ^{10}B atom for incident slow neutrons, thus fewer uranium atoms are required. Calculations indicate that for an anti-tumor antibody-directed approach, 30 to 200 uranium atoms are required to be conjugated to each antibody, as opposed to 300-1000 boron atoms. A recent breakthrough at BNL has achieved attachment of 300 to 1000 uranium atoms per antibody with maintenance of immunoreactivity. Funds are requested to carry this work further by developing a uranium assay system and use it to determine biodistributions of uranium coupled to anti-human tumor antibodies in mice with human tumor xenografts. These in vivo studies will quickly and effectively explore the feasibility of this approach to uranium neutron capture therapy (UNCT). This proposed work could lead to significant advances in clinical radiation therapy.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: The specific purpose of the project is to evaluate the potential of using uranium isotopes in neutron capture for cancer therapy.

The background leading to this work involved:

1) A perception that U-235, for example, may be better (per atom) than B-10 for neutron capture therapy.
2) The preliminary development of a method to attach 800 uranium atoms to one antibody, which by calculations may be sufficient for effective therapy.

The long-term objectives once this preliminary evaluation is completed are:

1) Escalate to full use of U-235 to demonstrate efficacy (i.e., tumor reduction in mice;
2) If warranted proceed on to phase I human trials.

TECHNICAL PROGRESS AND RESULTS: The uranium neutron capture therapy (UNCT) investigated in this project is centered around a previously developed method of linking 800 uranium atoms per one anti-human tumor antibody molecule. Testing of this particular strategy involves

the following steps:

1. Improving the serum stability of the uranium-antibody conjugate so that it is stable in vivo for the required time (e.g., 4-8 days).
2. Testing the immunoreactivity of the uranium-antibody conjugate in vitro to ensure that it still binds well to tumor cells and not to non-tumor cells.
3. Testing the behavior in vivo of the uranium-antibody conjugate to demonstrate its localization on human tumors implanted in mice. Biodistributions will be measured.
4. Development of accurate and sensitive uranium measurement methods. Implicit in most of the above work is the measurement of uranium concentrations. Since this is not particularly routine (chemical methods are too insensitive), methods must be developed to prepare samples for suitable measurement techniques (neutron activation, mass spectrometry, and electron microscopy) and the instruments themselves must be calibrated and lower limits of detection (and errors) determined. Several uranium isotopes must be measured, e.g., U-235 and U-238.
5. Further calculations will be made to more accurately determine the theoretical effectiveness and advantage of using, e.g., U-235.
6. U-238 (natural uranium) will be used for initial experiments whereas U-235 (useful for actual NCT) will be substituted later for NCT experiments.

TECHNICAL PROGRESS AND RESULTS:

1. Improved stability of uranium-antibody conjugates. It was found previously that at 4°C the uranium-antibody conjugate was very stable (virtually intact) after 10 days. However, it was found that at 37°C, uranium leaked out of the carrier protein after 24 hours. Therefore, other chemical modifications had to be made to stabilize it. Experiments included chemical and photo crosslinking, formation of other uranium compounds, and chemical reduction of the uranium. Almost all of these improved stability to 1-3 days but we are still searching for methods to extend this to the desired 4-8 days.
2. Serum stability studies were carried out at room temperature measuring at 0, 24 and 96 hours. These showed little breakdown or loss of uranium.
3. Cell binding (in vitro) of the uranium-antibody conjugate to human tumor and non-tumor cells was carried out. This was very encouraging in that selective and high binding of uranium to target tumor cells only was observed.
4. Two separate in vivo experiments were conducted injecting uranium-antibody conjugates into nude mice in human tumor implants. These were less successful at this stage due to the known breakdown of the preparation used (loss of uranium) at 37°C. Most of the injected uranium was found in the liver.
5. Development of analytical methods to measure U-238 content of samples. The appropriate instrument alterations, the handling of samples, the striving to reduce background, and the testing of calibration samples were involved. a) Inductively coupled plasma mass spectrometry

(ICPMS) was found to be useful down to ~0.1 ppb even though it is accurate to ppt levels. However, the method requires sample homogenization. b) Delayed gamma neutron activation of U-238 was carried out using the Brookhaven Medical Research Reactor (BMRR) and the HFBR. The procedure developed, which includes sealing the samples in ultrapure quartz tubes, provides ~1 ppb U-238 sensitivity when used with a cylindrical Ge detector. An anti-Compton shielded Ge well counter, which will be available in the near future, will provide ~10-fold improved sensitivity.

PAPERS/JOURNALS/PUBLICATIONS:

1. Uranium loaded apoferritin with antibodies attached: Molecular design for Uranium Neutron Capture Therapy. Proc. of the Nat. Acad. of Sci. (PNAS).In press.

PATENT:

1. Loading of Cavity Biostructures. Patent submitted to U.S. Patent Office. J. Hainfield, inventor.

NOTE: This project does not involve human subjects. This project does involve vertebrate animals.

PROJECT NUMBER: 92-22

PROJECT TITLE: Tunneling Microscopy Studies of Nanoscale Structures

PRINCIPAL INVESTIGATOR: Ocko, B.

LDRD FUNDING:

FISCAL YEAR		AMOUNT
1990		$ 0
1991		0
1992		84,860
1993	(authorized)	87,000
1994	(estimated)	0
TOTAL		$171,860

PROJECT DESCRIPTION:

It is hoped that this Laboratory Research Directed Research and Development grant will provide the seed money to initiate a strong program in scanning probe microscopy in the Physics Dept. There will be two parallel thrusts in the program. One will be to look at the tunneling of electrons into nano-structures. This work will be done in collaboration with Prof. Likharev of SUNY-Stony Brook. The second project involves tunneling in solutions and imaging the solid surface during electrochemical deposition as well imaging nanopores in artificial channels.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: In recent years there has been a revolution with the development and application of the tunneling microscope to areas of condensed matter physics. In this present work the microscope will be applied to two frontier areas. One involves the study of Coulomb blockade effects by tunneling at low temperatures into small particles, and the second experimental areas involves work on the solid/liquid helium interface. Both to image surfaces under electrochemical conditions and also to study the flow of ions through pores. This general area of surface structures in solution is related to the program in x-ray diffraction studies of the solid/liquid interface. The expertise in using the tunneling microscope will be of great value in the proposed program on nanochannels.

APPROACH: The approach has been to start the tunneling measurements in small particles in collaboration with Prof. K. Likharev of SUNY-Stony Brook and the electrochemistry work is being done in collaboration with workers in the Department of Applied Science. A tunneling apparatus that works at liquid helium temperature has been constructed, and an electrochemical microscope has been purchased between the Surface Physics and X-ray Scattering groups.

TECHNICAL PROGRESS AND RESULTS: We have been working on setting up a low temperature STM in BNL for the above research objectives. Our design effort has been concentrated on achieving the low noise level and large in-situ adjustment range of tip-sample separation. Therefore, we are able to achieve the low temperature surface atomic image and high resolution tunneling spectroscopy, as well as in-situ

sample preparation.

Our STM design is based on a new type of piezo electric motor which brings the tip to the sample from a macroscopic distance. A piezoelectric translator used in an STM can hold the tip-sample separation with sub-angstrom precision. Normally, the maximum adjusting distance for such a translator is a few microns. A mechanism to bring the tip-sample separation from a macroscopic distance into tunneling is crucial for STM design. For an STM operated at low temperature, a satisfactory approach has not been found. Low temperature STMs based on usual mechanisms (commercially available now) have generated many impressive surface topography and spectroscopy results. However, the problems of mechanical isolation and the small total adjustment range make such an approach unsuitable for our purpose. The various electric motors successfully incorporated in room temperature STMs do not work satisfactorily when subject to temperature change. The new motor we are using relies on the arrangements and utilization of friction forces between the piezo actuators and object being moved. The spring clamping between relative moving parts makes it less vulnerable to temperature change.

Our piezo electric motor can move the STM head (the scanner and the tip) to a mounted sample up to one centimeter away. This long driving distance makes easy in-situ sample manipulation, i.e. thin metal film deposition and sample cleaving. This electric motor approach makes complete vibrations through a few electrical leads. The size of the STM is 0.75 inches in diameter and 1.5 inches in length. It is very easy to adapt to various cryogenic apparatus, especially for sub-kelvin operation. At this moment, we have tested our STM on graphite samples from room temperature down to liquid helium temperature. The preliminary results show well ordered graphite atoms (down to 5.1 K) and indication of "Coulomb staircase" tunneling spectroscopy (77 K). A mini-evaporation source mounted directly on the STM head has been designed and tested at 77 K, and will be used for in-situ deposition of gold films on graphite. This novel setup gives us a great opportunity to create and observe controlled mesoscopic structures.

NOTE: This project does not involve human subjects. This project does not involve vertebrate animals.

PROJECT NUMBER: 92-27

PROJECT TITLE: Nuclear Techniques for Study of Biological Channels

PRINCIPAL INVESTIGATOR: Lynn, K.

LDRD FUNDING:

FISCAL YEAR	AMOUNT
1990	$ 0
1991	0
1992	68,398
1993 (authorized)	70,000
1994 (estimated)	0
TOTAL	$138,398

PROJECT DESCRIPTION:

The purpose of this project is to apply the instrumentation of high energy physics to the study of ionic channels in biological membranes. The signal by which we study ionic channels is rectangular current pulse, with unknown rise time (<10 μsec), ranging in duration from the minimal resolution time to many seconds, and in amplitude from immeasurable (i.e. less than 1 pA) to nearly 1nA. Such signals share characteristics with the signals routinely detected in high energy physics. In a crude sense, many of the particles of physics are also pulses of current, as seen by a detector. Thus, we propose to apply for the first time two standard technologies of high energy and nuclear physics to recording ionic channels.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: The purpose of this project is to advance the patch voltage clamp technique for the study of the picoampere range currents flowing through single ionic channels in the cell membrane. The techniques of Physics and Physiology are applied to the project. Two broad areas of instrumentation are addressed, i.e., 1.) automated analysis of single channel data, and 2.) reduction of noise arising from the electronics and electrodes etc. of the patch clamp; the available bandwidth of the electronics will also be increased. In addition, we have begun to use improved techniques developed to measure the time required for transitions between the open and closed conformation of a channel protein. We believe that such data will provide valuable information about the molecular events associated with open-close transitions and place important constraints of structural modeling of the channel protein.

APPROACH: Real Time Analyzer. Analysis of the open-close kinetics of single ionic channels is presently carried out after-the-fact by first digitizing the data and subsequently analyzing digitized records with automated or more often semi-automated software. This process is very time consuming, requires large amounts of data storage, and means that in general several days pass between gathering data and seeing meaningful results. We have begun to develop a Real Time Analyzer (RTA) which will detect open-close transitions and perform the

analysis of data as it is collected. It will distill the incoming stream of data into amplitude and duration information thereby vastly reducing data storage requirements and, more importantly, it will present meaningful results as the data is collected.

The software is a MS-Windows based application. The interface from the computer to the hardware is via GPIB bus. All the settings in the hardware are placed by the software, through different levels of menu-driven commands. The software also is responsible for correcting the base level drift and reading the collected data. These activities are all interrupt driven to insure that no loss of data would ever happen. In the time-slice in between, the software will histogram the data in a way preset by the user, and graphically plot them on the screen. When running with the stimulation cell voltage, the software traces the transit waveform first. The final offset transit waveform can be either the one averaged over a number of recorded transit waveforms or drawn by the user using the drawing capability in the software, or both. Possible data analysis will be added to the software.

Low Noise Electronics and Improved Electrode Technology. Noise is arguably the single greatest limitation of the patch voltage clamp technique. Since the signals to be measured are small - often less than 1 pA - and the duration of single channel events is usually very brief, measurements require extremely low levels of noise at relatively large bandwidths. We believe that it is possible to reduce the noise at a given bandwidth by factor of 3-4 below the best levels that are presently achieved. Such a reduction in noise can also be used to significantly extend the bandwidth (and thus improve time resolution) while preserving adequate signal to noise ratio. To achieve these reductions of noise requires attention to every detail of the technique. We will develop electronics with greatly reduced noise and are investigating various materials (selected for low dielectric loss) for the construction of input connectors, electrode holders, and the electrodes themselves. In the frequency range of greatest importance to most patch clamp recording situations (DC to about 20 kHz), dielectric noise from the electronics, connectors, holders and electrodes is a very important - often dominant - noise source. We are theoretically and practically addressing this source of noise.

Channel 'Rise time' Measurements. For these measurements we have selected a channel (VDAC) with an extremely high open state conductance; single channel currents larger than 1nA can be measured. At the highest bandwidth which allows an adequate signal to noise ratio, we measure the rise time of current at open-close and close-open transitions. This rise time is compared to the system rise time; if measurable differences can be resolved they can be attributed to the time involved in the channel protein molecule undergoing the conformational changes involved in gating. Ultimately we believe that we will be able to use bandwidths of 1-2 MHz in these measurements (about 50 times greater than used previously) and achieve a time resolution of about 100 nsec. If our time resolution is adequate to measure the phenomenon, we will investigate a variety of experimental interventions. This will be the first real attempt of measuring the rise time of a cell opening.

TECHNICAL PROGRESS AND RESULTS: Real Time Analyzer. Two prototype versions of the RTA have been developed during the past year. Work on the second of these is in progress at the time of this

writing. The feasibility of this project has been conclusively verified and most of the required software has been developed. However, a considerable amount of work remains before the protype devices produced so far will become practical laboratory instruments. Our present prototype can detect up to seven different current levels and continuously analyze data at a rate of 100,000 events per second with a 1 μsec time resolution. A third prototype is nevertheless required to incorporate various enhancements. Eventually, we will investigate edge-detection to identify channel events and a digital-signal-processor based instrument. This system will possibly become a laboratory standard in typical cell studies.

Low Noise Electronics and Electrode Technology. Theoretical work on both the origins of noise sources and how to reduce them have been accomplished. Several JFET input stages have been tested and preliminary results are promising. We have also begun development of a cryogenic input stage which will be cooled to temperatures in the range of 80-150°K. The Joule-Thompson effect cooler being used will meet the practical needs of the patch voltage clamp technique. Of particular significance are our investigations of dielectric noise arising from packaging, capacitors, and from the FET itself. Our investigations have shown both theoretically and experimentally that the usual rule of matching the FET input capacitance to the detector capacitance is not always valid, particularly when relatively low bandwidths (up to 10-50 kHz) or long shaping times are employed. We (R. Levis and K. Lynn) are finishing a manuscript describing these results.

Channel Rise time Measurements. We have gathered preliminary data with a bandwidth of 300 kHz, providing a time resolution of 0.5 -1 μsec. This bandwidth is not adequate to accomplish many of our goals and will be extended as lower noise electronics and techniques are developed. There has been some indication of detecting a measurable rise time however this needs further examination to determine the full extent of the measurement.

NOTE: This project does not involve human subjects. This project does not involve vertebrate animals.

PROJECT NUMBER: 92-28

PROJECT TITLE: RF Sources for Accelerator Physics (CAP)

PRINCIPAL INVESTIGATOR: Palmer, R.

LDRD FUNDING:

FISCAL YEAR		AMOUNT
1990		$
1991		
1992		98,792
1993	(authorized)	100,000
1994	(estimated)	0
TOTAL		$198,792

PROJECT DESCRIPTION:

The objective of this LDRD Program at BNL is to determine the practicability of a new type of klystron (Cluster Klystron) with possible application as a power source for Linear Colliders. The work would be done in collaboration with SLAC. The Cluster Klystron would have an efficiency, including that of the power supply, more than a factor of two higher than that expected of conventional tubes with the same specification (180 MW pulses of 600 nsec duration at 11.4 Mhz). In addition, the cluster tube would avoid the damage problems that have currently prevented the conventional tubes from operating other than at very low power levels or very short pulses. However, some basic questions must be answered before it is known if the device is fully practical:

1. Can a 40 AMP/sq cm thermionic cathode be run reliably, in a klystron, for a suitable life time (circa 10,000 hours)?

2. Is the electron beam generated from a magnetron type gun sufficiently free from instabilities and noise to allow its use in a klystron?

3. Can the mod anode in a magnetron gun be used to switch a 400 kV dc supply without problems of breakdown?

The proposed exploratory program would be aimed at answering these questions. The funds would be used to:

a. do the engineering and design and make the needed modifications to convert the existing SLAC Lasertron Power Supply and High Voltage Tank (to be provided by SLAC) into a test stand for a cluster klystron at BNL;

b. test a magnetron gun using the Varian conical cathode, to determine the noise of the gun design and lifetime of the cathode;

c. test the ability to switch the 400 Kv supply using a control pulse on the mod-anode of the magnetron gun.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: The proposed experiment involves the fabrication of a single channel

of a klystron that would eventually involve many such channels, all enclosed in a single large solenoid. The device will run from a dc source with internal switching using a pulsed modulated anode. It will use a magnetron type gun and a conical high current cathode already developed for the device by Varian.

APPROACH: The first phase of the experiment is to build and test the magnetron type gun. This has already begun.

A post graduate engineer has been hired. He is currently working full time on the project.

TECHNICAL PROGRESS AND RESULTS: A location for the experiment has been found in an experimental hall of the Tandem Van de Graaff. The area has been cleared and our equipment is already in place there. Electrical power for the magnet has been brought to the room. A preliminary safety analysis has been written and is under study.

Several pieces of used equipment have been obtained and brought to the hall. This includes:
One 400 kV dc power supply (loaned by SLAC)
One high voltage cable (")
One high voltage SF6 tank
Three large insulators
One 8-in bore 2-ft long 5Kg solenoid
One power supply for the magnet (from AGS)

In addition supporting materials and supplies have been purchased. The overall layout of the experiment has been specified. Engineering design has started, and some repair and modification of the used equipment has begun.

NOTE: This project does not involve human subjects. This project does not involve vertebrate animals.

PROJECT NUMBER: 92-29

PROJECT TITLE: Induction and Repair of Double-Strand Breaks in the DNA of Human Lymphocytes

PRINCIPAL INVESTIGATOR: Bender, M.

LDRD FUNDING:

FISCAL YEAR		AMOUNT
1990		$ 0
1991		0
1992		132,861
1993	(authorized)	0
1994	(estimated)	0
TOTAL		$132,861

PROJECT DESCRIPTION:

Ionizing radiation kills, mutates and transforms mammalian cells. The radiation induces many DNA-base changes, single-strand nicks and double-strand breaks. The latter are thought to be potentially lethal for cells. Variation among people in repair of double-strand breaks may be important to parameter to correlate with cytogenetic effects and to access risks in exposed populations. We propose to assay the induction and repair of such breaks in the genomic DNA of peripheral blood lymphocyte samples from volunteer BNL subjects.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: Among the various lesions induced by ionizing radiation in cellular DNA, perhaps the most important is the double-strand break (DSB). DSB manifest themselves as chromosomal aberrations, which are involved in cancer induction, and are strongly implicated as the primary cause of radiation-induced cell death. Such lesions are enzymatically repaired in human cells, but at least one human genetic disease, ataxia telangiectasia, appears to involve deficient DSB repair and to confer extraordinary radiation sensitivity on homozygotes. Should less extreme DSB repair competence variation occur in the normal human population, it would have important implications for radiation hazard assessment and the setting of exposure standards.

DSB and their repair overtime have traditionally been measured by density gradient ultracentrifugation or by neutral filter elution. These methods are tedious and yield rather erratic results. For testing the proposition that there is significant variation in DSB repair capacity in populations, a quicker and more reproducible technique is needed. Dr. John Sutherland of the BNL Biology Department has developed equipment which will accurately determine average molecular weight of DNA pieces from DNA samples using agarose gel electrophoresis. We have been developing quick and reproducible methods for the extraction of suitable DNA samples from human peripheral blood lymphocytes suitable for analysis by this method.

APPROACH: We have experimented with a number of methods to extract DNA from

separated lymphocytes using various combinations of detergents, proteinase and RNA se with phenol-chloroform or inorganic purification steps. These suffer the disadvantages that the DNA is in very large (megabase) pieces and that the lymphocytes must be separated from the whole blood before extraction begins. The former is a disadvantage because the DNA is too large to run in simple agarose gels, and hence must either be cut with restriction enzymes or an added dose of ionizing radiation before loading or else must be run for long times with pulsed field electrophoresis schemes.

We are now evaluating an alternative method which used the cationic detergents dodecyltrimethylammonium bromide and cetyltrimethylammonium bromide for cell lysis and selective DNA precipitation. There are two advantages: the DNA (for unexplained reasons) is of a quite uniform size class at around 200 KB, and the method works on whole blood samples, eliminating the need to separate lymphocytes.

TECHNICAL PROGRESS AND RESULTS: We have evaluated several commercial kits implementing this method as well as our own protocols. We have found it necessary to add a proteinase step in order to remove protein crosslinked to DNA in irradiated samples, and are presently evaluating samples from irradiated cells to see what modifications, if any, may be necessary to get reliable precipitation of the DNA by the cetyltrimethlyammonium bromide at low salt concentration. We have evaluated various sorts of agarose and time/voltage possibilities and now routinely achieve good, reproducible gel electrophoresis runs. Gels have been analyzed with Dr. Sutherland's device, and though not enough has been done so far to properly analyze variability in the molecular weight determinations, the results appear quite reasonable.

NOTE: This project does involve human subjects. This project does not involve vertebrate animals.

PROJECT NUMBER: 92-30

PROJECT TITLE: An EBIS Source of High Charge State Ions up to Uranium

PRINCIPAL INVESTIGATOR: Alessi, J.

LDRD FUNDING:

FISCAL YEAR	AMOUNT
1990	$ 0
1991	0
1992	61,548
1993 (authorized)	66,000
1994 (estimated)	0
TOTAL	$127,548

PROJECT DESCRIPTION:

The objective of this project is to gain the necessary experience in a new area of applied science and technology, and to prove that the design of an Electron Beam Ion Source (EBIS) for RHIC applications is feasible. The project will concentrate on the development of a prototype source of heavy ions, capable of satisfying present and future programs. An EBIS of high charge heavy ions, coupled to a radiofrequency quadrupole accelerator (RFQ) and a superconducting linac, has long been considered. Such a system would offer many advantages; a spectrum of all ions up to uranium, a possibility to allow for future needs for a higher luminosity, and a simpler and more reliable operation requiring less maintenance, with substantially reduced power.

We propose to refurbish and modify an EBIS with a superconducting magnet and from its test performance with several ion species, including uranium, test the scaling laws for EBIS sources. These experiments and analysis will serve as a basis to eventually design an EBIS with parameters compatible with machines, such as RHIC. As a basis for this project we will use an almost complete superconducting EBIS, which we obtained from Sandia Laboratories on a long-term loan. The support requested will cover the fabrication of the stand to mount the source, a minor repair of the superconducting magnet, fabrication of several components for the source, the salary for six months for the Sandia scientist who designed the source and who will come to BNL to help with the assembly, and the costs to operate the source.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1992:

PURPOSE: In this project, we will gain experience in the design and operation of an EBIS for production of high charge state heavy ions. We will study scaling laws for ion production in an EBIS. From these results, we will then determine if this type source could fulfill the requirements of an injector for RHIC. Long term objectives, if initial results are promising, would be to study improvements to the design of such a source to obtain higher ion intensities. These could include improvements to the electron beam intensity and studies of performance with ion injection into the EBIS. This could ultimately lead to a

proposal for a new injector for RHIC, in which an EBIS - RFQ - superconducting linac combination could serve as a compact injector for heavy ions, located very close to the Booster.

APPROACH: What made this project feasible was the fact that we were able to obtain on long term loan from Sandia Laboratories, most components of a state-of-the-art, superconducting EBIS. Although several million dollars were invested in the development of this source at Sandia, it was never assembled due to the cancellation of the associated atomic physics program. Our strategy is to fabricate the remaining components required for the source, assemble and test it in stages, and then proceed with experiments using the source. Although originally designed to produce low intensities of almost fully stripped heavy ions for atomic physics experiments, we plan to operate the source in a regime more suited as an injector, attempting to produce higher intensities but lower charge states (ex. U^{46+}). This relaxes some of the parameters of the source - lower magnetic fields, lower electron beam energies, and less stringent vacuum requirements. Therefore, parts of the source are being modified to better suit our program.

TECHNICAL PROGRESS AND RESULTS: The final assembled source, with associated power supplies, will require approximately 800 square feet of floor space. Therefore, following project approval, we first had to move several ion source test stands out of the Building 930 extension to make lab space. EBIS source hardware, most of the required power supplies, and the vacuum components were shipped from Sandia in dozens of large shipping crates. These crates were stored in the basement of the 200 MeV linac at BNL. Another early task was therefore the uncrating and organization of all source hardware and mechanical drawings. (These were more or less randomly packed in the shipping crates). All drawings have now been filed, and most of the source components have been removed from the crates, identified, and stored on shelves in the lab. We are in the process of checking hardware against prints in order to identify any missing components which we will have to fabricate.

The original stand for the source had been excessed by Sandia, so a new stand is being fabricated in the BNL shops, and should be delivered by the end of October. The superconducting magnet is 1.2 m long, has a 15 cm diameter cold bore, and reaches fields of approximately 4.5 T with a straightness of 1 part in 10^4, and a uniformity of 1 part in 10^3. This magnet has a system which allows it to be precisely aligned relative to the electron beam while the source is in operation. The superconducting coil can be moved within the cryostat by adjusting 8 support rods. It was known that several of these support rods were broken while at Sandia. Once the magnet was placed on a temporary stand at BNL, we opened up the cryostat and found 6 broken support rods. New rods were fabricated and installed. Presently, we are identifying all temperature sensors in the magnet, and repairing broken and mislabeled wires. Once the permanent stand is delivered, we will mount the magnet and energize it before proceeding with the rest of the source assembly.

Regarding other aspects of the source, we are preparing to submit the drawings to the shops for the fabrication of the electron gun. We have done magnetic field calculations to map the fringing fields of the superconducting magnet (unshielded), and are looking at various options for shielding or removing the ion beam diagnostics from

these fringe fields. We have also developed the capability of doing the electron and ion beam optics calculations, and are presently modelling the electron optics from the gun, through the magnet and into the electron collector.

Dr. R. Schmieder, the Sandia scientist who designed the source, came to BNL in September for the first time since we had received the source. With him, we verified that we had correctly identified components, and discussed plans for the source assembly sequence.

NOTE: This project does not involve human subjects. This project does not involve vertebrate animals.

END

DATE FILMED

5/3/93