BNL--52320

DE92 009018

Laboratory Directed Research & Development Program

**Annual Report to the Department of Energy
December 1991**

**Gregory J. Ogeka
Special Assistant to the
Associate Director for Administration**

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

UNDER CONTRACT NO. DE-AC02-76CH00016

UNITED STATES DEPARTMENT OF ENERGY

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

NTIS price codes:
Printed Copy: A04; Microfiche Copy: A01

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INTRODUCTION

Today, new ideas and opportunities, fostering the advancement of technology, are occurring at an ever-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.

**LABORATORY DIRECTED RESEARCH & DEVELOPMENT PROGRAM
FISCAL YEAR 1991**

SUMMARY OF PROJECTS

PROJECT NO.	PROJECT TITLE	AMOUNT
90-27	Small Gap Undulator for X-ray Ring	\$ 100,232
90-41	Advances in Geoexploration	99,709
90-44	Transvenous Coronary Angiography with Synchrotron X-Rays	29,043
90-45	Borehole Measurements of Global Warming	34,913
90-46	Modeling of Time Evolution of Climate Systems	73,000
90-47	Oceanic Carbon Dioxide Sinks	89,306
90-48	Cloud and Aerosol Albedo Studies	52,399
91-03	Molecular Ecology: Development of Field Methods for Microbial Growth Rate and Activity Measurements	49,229
91-04	A New Malaria Enzyme - A Potential Source for a New Diagnostic Test for Malaria and a Target for a New Antimalarial Drug	52,763
91-07	Basic Studies on Thoron and Thoron Precursors	37,941
91-08	Cloning of the cDNA for a Human Serine/Threonine Protein Kinase that is Activated Specifically by Double-Stranded DNA	85,318
91-09	Combined Boron Neutron Capture Therapy (BNCT) and Adoptive Immunotherapy in the Treatment of Experimental Brain Tumors	34,869
91-10	Feasibility Study for Microbeam Radiation Therapy with 30-90 keV X Rays from the NSLS X17 Beamline	60,742

**LABORATORY DIRECTED RESEARCH & DEVELOPMENT PROGRAM
FISCAL YEAR 1991**

**SUMMARY OF PROJECTS
(Continued)**

PROJECT NO.	PROJECT TITLE	AMOUNT
91-11	Proposal for Development of Deposition Methods for Micromechanics	76,483
91-15	Photon Data Automation	29,076
91-18	Development of an Ultra-Fast Laser System for Accelerator Applications	79,657
91-19	Cluster Impact Fusion	29,696
91-20	A Sequence Related to the Human Gonadoliberin Precursor Near the N-Termini of HIV and SIV GAG Polyproteins (Gonadoliberin Precursor Momicry in in HIV P-17: AIDS Vaccine and Antiviral Peptides)	54,998
91-21	Effect of a Bacterial Spore Protein on Mutagenesis	87,461
91-22	Development of Superferric Micro-Undulators	100,001
91-23	Structure and Function of Adenovirus Penton Base Protein	64,623
	TOTAL EXPENDITURES	\$1,321,459

LABORATORY DIRECTED RESEARCH AND DEVELOPMENT FUNDED PROJECTS BY DEPARTMENT FISCAL YEAR 1991

<u>Department</u>	<u>Amount (\$000)</u>
Applied Science	399
Biology	290
Chemistry	147
Instrumentation	76
Medical	180
Nuclear Energy	29
Nat'l Synchrotron Light Source	<u>200</u>
Total	\$1,321

PROJECT NUMBER: 90-27

PROJECT TITLE: SMALL GAP UNDULATOR FOR X-RAY RING

PRINCIPAL INVESTIGATOR: Stefan, P. M.

LDRD FUNDING:	FISCAL YEAR	AMOUNT
	1990	\$ 49,853
	1991	<u>100,232</u>
	TOTAL	\$150,085

PROJECT DESCRIPTION:

This project proposes to investigate problems related to the development of a variable-gap vacuum chamber for a prototype small-gap undulator, intended for the X-ray Storage Ring. Ultimately, solution of these problems could enable installation of a prototype small-gap chamber and magnet in the X-ray Ring with no effect on the stored beam lifetime, for a full vacuum gap of as little as 2 mm. The major problem areas are related to a minimization of the rf impedance of the structure while preventing ion trapping and build-up of a high pressure region in the small gap. Investigations will include: 1) designs and tests of reliable, movable vacuum seals which do not produce gas bursts during movement, 2) optimization of pumping speed in the small gap, 3) possible incorporation of effective ion clearing electrodes, 4) design of compatible shields, resistors, and transitions to decrease the rf impedance, 5) impedance measurements of structure mock-ups and prototypes.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1991:

Early in FY91, design was completed of a minimum-surface-area movable vacuum seal, a single-convolution bellows with a race-track form, only to fail in having it fabricated. Neither commercial vendors nor in-house shops would attempt it. We immediately changed our design approach to one based on known commercial capabilities and arrived at a new design, a multi-convolution, hydroformed-type bellows of a circular form. The bellows material thickness was chosen at a compromise between maximum bellows service life and reliable electron beam welding of the bellows into subsequent assemblies. Five test bellows have been fabricated.

Also in FY91, the capacity was built up to make rf beam impedance measurements of accelerator components. A system of calibration standards and adapters was designed, built, and interfaced to a vector network analyzer. Initial measurements were made on a small hydroformed-type bellows.

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	<u>60,000</u>
	TOTAL	\$248,136

PROJECT DESCRIPTION:

Useful advances in geoexploration include the confirmation of the validity of simple biomarkers for hydrocarbon source identification, such as sulfur isotopes, 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.

PROJECT NUMBER: 90-41 (continued)

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1991:

PURPOSE: Useful advances in geoexploration include the confirmation of the validity of simple biomarkers for hydrocarbon source identification, and the development of techniques for the analysis of hetero compounds in complex hydrocarbon mixtures. This work concentrates on the development of methodologies for the speciation of sulfur compounds and other light element compounds in complex hydrocarbon mixtures such as crude oils, kerogen, and organic sediments.

APPROACH: X-ray absorption spectra are taken in Beamline X-19a at the NSLS. Samples are scanned in the neighborhood of the absorption edge with a relative energy bandwidth of 2×10^{-4} . At high concentrations of the target element, the scan is performed by determination of the absorbance, whereas at trace concentrations the spectra are measured by observation of the elemental fluorescence. For analysis of the EXAFS spectra the periodic nature of the absorption with changing excitation energy can be analyzed to yield crystallographic data of the volume around the absorbing element. For analysis of the XANES regions of the spectra, comparisons are made with spectra taken from model compounds of that element.

TECHNICAL PROGRAMS AND RESULTS: In 1991 all experiments used the low energy setting of Si-111 crystals, and all work was done at the sulfur K edge. XANES spectra were collected for standards, mixtures of known sulfur compounds in a simulated sediment matrix, field samples of sediment from a variety of cores, laboratory samples from experiments studying the rate of oxidation of sulfur compounds, oils exposed to varying degrees of biodegradation, and oils and coals both untreated and biodegraded in the laboratory with various strains of bacteria.

Several modifications were made at X19a to extend the capability of the beamline for light element analysis. These include the development of a diamond window to increase available flux, and to permit the penetration through the window of fluorescence photons from elements lighter than sulfur. The development of crystals for light element detection is also under way.

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.

PROJECT NUMBER: 90-41 (continued)

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

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 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.

PROJECT NUMBER: 90-44

PROJECT TITLE: TRANSVENOUS CORONARY ANGIOGRAPHY WITH
SYNCHROTRON X-RAYS

PRINCIPAL INVESTIGATOR: Chanana, A. D.

LDRD FUNDING:	FISCAL YEAR	AMOUNT
	1990	\$41,070
	1991	<u>29,043</u>
	TOTAL	\$70,113

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-rays 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 much more x-ray fluence and a new dual-detector system will be utilized, an increase in available x-ray flux by a factor of 25 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 1991:

A total of six human studies (19 imaging procedures) have been performed in the transvenous coronary angiography project. The imaging procedures have been performed in the Synchrotron Medical Research Facility (SMERF) at the National Synchrotron Light Source. The project made great advances during the period of the LDRD funding in the areas of instrumentation development, understanding the human imaging parameters, and in the quality of the coronary arterial images.

PROJECT NUMBER: 90-44 (continued)

The human studies reported here were all part of the validation phase of the program. The studies were designed to determine: 1) what views are possible and the optimal viewing angles; 2) which parts of the coronary arterial trees are visible with good image contrast; 3) what injection parameters are optimal for the iodinated contrast agent (Angiovisc 370), i.e. injection rate and injection volume; 4) the effects of beam energy harmonics (due to various photon spectra from the superconducting wiggler magnet) on the image quality; 5) the effects of varying the patient scan rate.

A detailed analysis of runs at fields ranging from 3.0 T to 4.4 T shows that harmonics in the beam are not a significant problem. Analysis routines have been developed to deal with the harmonic artifacts and improvements in the beamline hardware have greatly reduced harmonic contamination. It was also determined that arterial motion artifacts appear far more significant at a scan speed of 6 cm/sec than at the standard 12 cm/sec. Future data will thus be taken at 12 cm/sec and a wiggler field as high as operations permit.

Of the six patients, excellent images of the Right Coronary Artery (RCA) were obtained from four, and the Left Anterior Descending (LAD) from one of these. The remaining two patients were too thick, thus attenuation of the beam reduced the signal/noise ratios to an unacceptably low level. The optimal injection parameters for viewing the RCA are 36 ml at 15 ml/sec. Viewing angles of 30° to 40° LAD give excellent projection of the RCA artery away from the ventricles. Optimal time between contrast agent injection and imaging varied from patient to patient. Attempts to visualize the LAD were not as successful. Optimization of the injection parameters and viewing angles were not completed. Future studies are designed to complete the optimization for the LAD.

A lot of progress was made in the development of computer software for the image processing. It is now possible to serially visualize successive frames in a cine-like mode, to quantify arterial cross-sections, to image coronary anatomy at a spatial resolution of 0.5 mm, and to understand the effects of beam harmonics and system alignment.

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	<u>54,000</u>
	TOTAL	\$89,001

PROJECT DESCRIPTION:

The objective of this research is to obtain temperature profiles with millikelvin precision by direct measurement in existing boreholes to determine temporal changes in local surface temperatures with resolution of a day (top few centimeters) to centuries (hundreds of meters of depth). This will provide a test of a hypothesis that warming has occurred and can be observed in geotemperature signatures. Resolution of surface temperature difference signatures decay exponentially with depth, but are sufficient to detect changes on the order of a degree over a period of decades to a century. Similar analyses made in Alaskan permafrost indicating surface warming of 2 to 4 degrees C over the past few decades to a century (Lachenbruch and Marshall 1986). Borehole data from Norway will be compared to extant data from Alaska. If the method is successful, then it may provide a means for validating GCMs by comparing their warming "predictions" of times past at various world location when the GCMs are parameterized with historical data.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1991:

PURPOSE: This research addresses an hypothesis that global warming can be measured directly. A putative mechanism driving warming is the observed increase in atmospheric CO₂. This driver is strongly regulated by CO₂ 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" variously including borehole measurements. We are investigating the links in this hypothetical chain initially by concentrating on understanding photosynthetic mechanisms regulating carbon uptake in a CO₂-enriched environment potentially leading to biomass changes.

PROJECT NUMBER: 90-45 (continued)

PROGRESS AND RESULTS: This project has been evolving. Initially some outright borehole measurements were contemplated. However, last May we saw a target of opportunity for a Chaired Professor, Dr. Stephen Long, to join us in investigating this terrestrial component of Global change, trying to understand the takeup of carbon by plants.

During FY 1991, Dr. Hendrey scoped out the program and began the investigation of what specifically should be done. There were several discussions with DOE headquarters to ascertain what they would be most interested in funding. Dr. Long was also brought here for a short period to help define the investigation.

We have now hired Dr. Long, an expert in the responses of plants to CO₂ enrichment and he will investigate the physiological and biochemical responses of plants to increased CO₂, CO₂ and temperature interactions, and the development of physiologically-based models of plant responses of the type that will be needed to evaluate changes in terrestrial surface cover.

PROJECT NUMBER: 90-46

PROJECT TITLE: MODELING OF TIME EVOLUTION OF CLIMATE SYSTEMS

PRINCIPAL INVESTIGATOR: Peirls, R.

LDRD FUNDING:	FISCAL YEAR	AMOUNT
	1990	\$ 65,201
	1991	<u>73,000</u>
	TOTAL	\$138,201

PROJECT DESCRIPTION:

The objective of this program is to improve methods of computing transient response of the coupled climate system to external forcing. A time dependent description of the coupled climate system is crucial for the prediction of the effect of anthropogenic activities upon the global environment. Currently available mathematical and computational tools for developing credible time dependent descriptions are inadequate. Improvement is needed in evaluating the sensitivity of the time to the different processes that couple climate subsystems (atmosphere, ocean, cryosphere, etc.). Equally important is the development of improved computational techniques to allow full and flexible utilization of models of these coupled systems.

The proposed work will consist of the following:

- Parameterized models of the coupled climate systems will be developed and exercised to investigate system properties.
- New computational methods for solving loosely coupled problems on distributed systems will be explored.
- The parameterized models and the computational techniques will be used to assess the viability of carrying out loosely coupled computations of full scale models.

If indicated by the initial studies, these techniques will be applied to running a full scale Global Change Model (GCM) coupled to an ocean model in order to explore climate response to anthropogenic forcing.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1991:

PURPOSE: This project was designed to address the need to improve both computational methodologies and process representations for the treatment of interacting subsystems that comprise the total climate system. Its focus was on ocean-atmosphere coupling, emphasizing the implementation of subsystems as independent program

PROJECT NUMBER: 90-46 (continued)

modules, interacting with one another only through their mutual effect on shared data. Provided that the individual modules have suitable time discretizations, the results of the coupled model should be only weakly dependent on the degree of synchronization between the modules. If this is true, then operation in a multiple computer environment should allow substantial improvement in climate model throughout.

APPROACH: The overall strategy was to use prototype models to evaluate computational methods for coupling models of climate subsystems in a range of computational environments. Highly parameterized models would be used to rapidly test the basic concept of loosely synchronized coupling. Mesoscale models would be used to understand in more detail the structure of the coupling in more realistic models.

PROGRESS AND RESULTS: Procedures for parallel operation of large-scale models were first tested with a regional scale primitive equation oceanic model driven by wind stress using multiple processors on a single supercomputer. The oceanic model was The Semi-Spectral Primitive Equation (SPEM) model developed by D. B. Haidvogel of Rutgers and it was run in multi-tasking mode on the NERSC Cray-2. The atmospheric calculation was a simple time-dependent computed wind field, not driven by the ocean. Since this starting calculation was in preparation for coupling to a more general atmospheric model, the winds were calculated relative to a standard North-South geographic grid. Transformation routines were added to the SPEM model for generalized conversions. Multitasking and single task versions of the model were run showing minimal differences.

Exploration of the potential of loose synchronization was carried out using a very highly parameterized prototype climate model. The atmosphere was treated by a globally averaged energy balance model in which the infra-red loss to space is determined by CO₂ concentration, and heat exchange takes place with the ocean. The ocean is described by a one-dimensional upwelling diffusion model. The CO₂ equation has a long term uptake by the ocean, driven by ocean temperature. There is an anthropogenic response equation that assumes the rate of at which CO₂ is generated will be reduced as a result of rising global temperature.

This model was run in several environments. First it was run as a single tasking computation on the NERSC Cray-2. Next, it was run in multitasking mode allowing a variable time lag between the different components. Finally, it was run using experimental software allowing coupled calculations to be distributed over a network.

PROJECT NUMBER: 90-46 (continued)

The results showed that differences of the order of ten time steps between one model component and the coupling information it receives from another, resulted in very small (of the order of 1%) changes in the predictions of the model.

SIGNIFICANT FINDINGS: While the models considered in this exploratory work were highly simplified, the results strongly suggest that successful large scale climate model calculations can indeed be carried out in a distributed computing environment using this loose coupling approach.

FOLLOW-ON FUNDING:

1. Coupling of Advanced Climate Model Component Subsystems, DOE/OHER AS-250-ASD, KP-05, FY 1991 \$248,000.

PROPOSALS:

1. A proposal entitled "Coupling of Advanced Climate Model Subsystems" was submitted to the Atmospheric and Climate Research Division, Office of Health and Environmental Research of the Department of Energy.

PROJECT NUMBER: 90-47

PROJECT TITLE: OCEANIC CARBON DIOXIDE SINKS

PRINCIPAL INVESTIGATOR: Minnett, P. J.

LDRD FUNDING:	FISCAL YEAR	AMOUNT
	1990	\$ 9,924
	1991	<u>89,306</u>
	TOTAL	\$99,230

PROJECT DESCRIPTION:

The objective of this project seeks to quantify increases in oceanic carbon fixation in the past century by examining historical records of upper phytoplankton abundance. Half of the anthropogenic CO₂ released to the atmosphere is apparently absorbed by the ocean. Although a large fraction of the missing carbon may be absorbed by physical-chemical diffusion across the air-sea interface, some fraction may be actively absorbed by phytoplankton photosynthesis, a so-called "biological pump." If the biological pump hypothesis is true then the standing stock of phytoplankton should have increased since the industrial revolution. This proposal seeks to investigate what changes, if any, have occurred in the standing stocks of phytoplankton in the central ocean basins over the past 100 years. The approach is based on the robust relationship between Secchi depths and surface chlorophyll concentrations.

The work to be done consists of the following:

- Acquire and bin NODC Secchi disc data.
- Analyze the time rate of change for statistical trend in the central ocean basins.
- Acquire new data from various marine labs around the world, as necessary.
- Repeat the analysis for the time rate of change.
- Calculate the rate of change in carbon fixation in the central ocean basins and quantify the biological pump.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1991:

PURPOSE: To establish a research program into Global Climate Change, in particular making use of satellite remote sensing over the oceans and ice.

PROJECT NUMBER: 90-47 (continued)

APPROACH: The fluxes of CO₂, heat and water between the oceans and atmosphere are critical parameters for the study of Global Climate Change. Earth observation satellites are an important tool for the generation of global and regional data sets needed for this research, and while these parameters are not directly measurable by satellite instruments, they can be related through models or physico-statistical relationships to those which are.

The main activities have included:

- forging links with groups working on satellite oceanography;
- undertaking a research cruise to validate satellite oceanographic retrievals (a six-week cruise in late 1991 on the R/V Alliance);
- simulating satellite infrared radiometer measurements using a line-by-line atmospheric radiative transfer model;
- analyzing existing measurements for satellite data validation;
- contributing to activities in the scientific planning of ARM.

PAPERS/JOURNALS/PUBLICATIONS:

1. Minnett, P.J., 'Consequences of sea surface temperature variability on the validation and applications of satellite measurements', J. Geophys. Res., 96,18,475-18,489.
2. Gautier, C., P.J. Minnett, L.R. Thorne, J. Tichler, J. Vitko and P. G. Weber. 'The possible use of satellite radiance measurements by ARM'. U.S. Department of Energy.
3. Minnett, P.J., D.T. Llewellyn-Jones, I.J. Barton, C.T. Mutlow, and A. M. Zavody, 'The Along-Tract Scanning Radiometer - an experimental satellite instrument for measuring sea-surface temperature for global climate research'. In preparation.

FOLLOW-ON FUNDING:

1. Diagnostic Analysis of Iron-Limitation of CO₂ Uptake by Oceanic Phytoplankton, DOE/OHER, AS-293-OASD, B&R KP-05, FY 1991 \$111,000.
2. Collaborative research on the Northeast Water Polynya: Water Mass and Air-Sea Interaction', to the Division of Ocean Sciences, NSF (as a subcontractor to North Carolina State University). \$96,000 in FY92, \$85,000 in FY93, and \$93,000 in FY94.

PROJECT NUMBER: 90-47 (continued)

PROPOSALS:

1. "Mid-Latitude Validation of AVHRR Infrared Radiances and Sea Surface Temperature Measurements", to the NOAA Climate and Global Change Program. In review. \$43,815 requested for FY92.
2. "Seasonal Heat Storage and Estimations of Ocean-Atmosphere Heat Exchanges and at High Latitudes from Remotely Sensed Measurements", with North Carolina State University, to the National Institute for Global Environmental Change. In review. \$41,318 requested for FY93.

PROJECT NUMBER: 90-48

PROJECT TITLE: CLOUD AND AEROSOL ALBEDO STUDIES

PRINCIPAL INVESTIGATOR: Schwartz, S. E.

LDRD FUNDING:	FISCAL YEAR	AMOUNT
	1990	\$ 72,303
	1991	<u>52,399</u>
	TOTAL	\$124,702

PROJECT DESCRIPTION:

This study will involve the following components:

- Examine satellite data for indication of enhancement of cloud albedo in regions influenced by sulfate aerosol derived from anthropogenic emissions.
- Calculate concentrations of sulfate aerosol as a function of space and time in regions where satellite measurements of cloud albedo are available.
- Calculate vertical profiles of aerosol particles by combining extinction measurements from solar occultation and downward looking satellites.
- Examine the influence of aerosol composition on cloud albedo.
- Assess from existing measurements the influence of organic and inorganic carbonaceous aerosol on planetary albedo--both direct influence and as modifier of cloud properties.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1991:

BACKGROUND: A persuasive argument can be made that sulfate-containing aerosol particles resulting from SO₂ emissions associated with fossil fuel combustion contribute to radiative forcing of climate by scattering incident shortwave (solar) radiation and by increasing the light scattering by clouds that form on these aerosols. This perturbation in radiative forcing is estimated to be comparable, on a global scale, to the perturbation in greenhouse forcing due to increased concentrations of anthropogenic infrared active gases (principally CO₂), but of opposite sign. Because of the hemispheric asymmetry in sulfur emissions one might expect to find evidence for the resulting albedo perturbation in interhemispheric comparisons of albedo or temperature anomaly. However no such evidence has been found so far in interhemispheric comparisons.

PROJECT NUMBER: 90-48 (continued)

APPROACH: This project represents a sensitive test of aerosol forcing by searching for cloud albedo perturbation directly in satellite data on small spatial and temporal scales. Specifically this project consists of an examination of the spatial and temporal dependence of albedo in existing satellite measurements at locations and times where perturbation is expected based on numerical modeling of sulfate aerosol concentrations attributable to anthropogenic SO₂ emissions. The time period 1986-87 was selected as the initial time period to search for a signature of anthropogenic sulfur emissions in cloud albedo data. During this time period the data of two Earth Radiation Budget Experiment (ERBE) radiometers orbiting on two different satellites are available as are the necessary meteorological data to drive the chemical transport model. The cloud albedo signal is expected to be most noticeable in low altitude clouds over ocean. The selection of low clouds from the ERBE data is based on the smaller temperature difference with respect to the surface than high altitude clouds. Identification of fully cloudy scenes is based on comparison of adjacent pixels.

ACCOMPLISHMENTS: Work to date has included acquisition and analysis of satellite data, acquisition and preparation of meteorological data, and model development. Satellite data are transferred to Sun workstations via high speed electronic link. Several computer programs have been written for identifying appropriate scenes and extracting subsets of the data, and for sorting, binning and displaying the data. Model development activities consist of compilation of emission inventories, development of chemical mechanisms, and development of radiation codes.

Dr. Richard Wagener joined this activity in 1990 under support from Exploratory Research Program funds. His principal activity has been analysis of the satellite data. He has adapted a spatial coherence algorithm developed by Coakley and Baldwin (1984) for AVHRR (Advanced Very High Resolution Radiometer) data to the identification of low altitude homogeneous cloud cover in ERBE data. Additionally Dr. Wagener has adapted the "Doubling and Adding" radiative transfer code of Hansen and Travis (1974) to evaluate cloud albedo as a function of cloud thickness for varying cloud droplet densities and liquid water content. Dr. Schwartz has collaborated with a number of investigators at other institutions in an examination of data pertinent to the aerosol forcing issue and identification of approaches that will be most fruitful in defining this forcing and describing it in climate models. A "white paper" (BNL 46786) resulting from this collaboration has been distributed to DOE and the other participating agencies of the Committee on Earth and Environmental Sciences (CEES), and a shorter version is scheduled for publication in Science in February 1992.

The following are the principal conclusions of the paper:

PROJECT NUMBER: 90-48 (continued)

- Anthropogenic aerosols exert a climate forcing that is comparable in magnitude to that of anthropogenic greenhouse gases but opposite in sign and having different spatial and temporal properties.
- It is likely that climate forcing by anthropogenic aerosols has offset global greenhouse warming to a substantial degree and is masking the onset of detection of the greenhouse effect of anthropogenic CO₂ and other greenhouse gases. However differences in geographical and seasonal distributions between the aerosol and greenhouse forcings preclude any simple compensation.
- It is mandatory to take aerosol effects into account in evaluating anthropogenic influences on past, current, and projected future climate and in formulating policy regarding controls on emissions of greenhouse gases and sulfur dioxide.
- Resolution of such policy issues requires integrated research on the magnitude and geographical distribution of aerosol climate forcing and on the controlling chemical and physical processes.

FUTURE WORK: The work initiated in this project will be continued under support from DOE. Principal components of this research are:

- Model calculations of the enhancement of cloud and clear-sky shortwave albedo and radiative forcing due to anthropogenic sulfate as a function of geographical location (ca. 2 degree resolution) and time (24 hour resolution) for four seasonal months in 1986-87.
- Literature review and data analysis of dependence of concentration of cloud drops on sulfate concentration.
- Evaluation of spatial and temporal patterns of anomaly (departure from temporal-mean values) of cloud and clear-sky shortwave albedo from archived satellite measurements.
- Examination of correlation of observed and calculated spatial and temporal patterns of calculated and observed cloud and clear-sky albedo anomaly.

An extension of this work will be to examine the spatial pattern of temperature anomaly in relation to the long-term spatial pattern of perturbation in shortwave radiative forcing due to anthropogenic sulfate to ascertain whether a climate linkage can be discerned in this way.

PAPERS/JOURNALS/PUBLICATIONS:

1. Cloud albedo and climate perturbations due to anthropogenic

PROJECT NUMBER: 90-48 (continued)

sulfate. Schwartz, S.E. GLOMAC (Global Modeling of Atmospheric Chemistry) Workshop, Schloss Ringberg, Tegernsee, Germany, January 28-February 2, 1990; BNL-43838. Invited presentation.

2. Climate Forcing by Anthropogenic Aerosols. Charlson, R.J., Schwartz, S.E., Hales, J.M., Cess, R.D., Coakley, J.A. Jr., Hansen, J. E., and Hofmann D. J. BNL-46786.
3. Climate Forcing by Anthropogenic Aerosols. Charlson, R.J., Schwartz, S.E., Hales, J.M., Cess, R.D., Coakley, J.A. Jr., Hansen, J.E., and Hofmann D.J. Science, in press (1992).

FOLLOW-ON FUNDING:

1. Cloud Albedo Perturbations on Climate. DOE/OHER, AS-248-ECD, B&R KP-05, FY 1991 \$620,000, FY 1992 \$630,000.
2. Radiation Properties on Non-Uniform Clouds. DOE/OHER, AS-279-ECD, B&R KP-05, FY 1991 \$240,000.
3. Atmospheric Radiation Measurements. DOE/OHER and thru Battelle PNL - FY 1991 \$711,000, FY 1992 \$650,000.

PROPOSALS:

1. A search for empirical evidence of the clear sky albedo perturbation due to sulfate aerosols using satellite radiometry and an Eulerian chemical transport and transformation model. DOE/National Institute for Global Environmental Change. Pending.
2. Monitoring Global Aerosol Distributions on the Earth and Mars. DOE/Space Exploration Initiative. Pre-proposal, pending.

PROJECT NUMBER: 91-03

PROJECT TITLE: MOLECULAR ECOLOGY: DEVELOPMENT OF FIELD METHODS FOR MICROBIAL GROWTH RATE AND ACTIVITY MEASUREMENTS

PRINCIPAL INVESTIGATOR: Kemp, Paul F.

LDRD FUNDING:	FISCAL YEAR	AMOUNT
	1991	\$ 49,229
	1992	<u>51,000</u>
	TOTAL	\$100.229

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 artifacts. 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 1991:

PURPOSE: Existing methodologies for measuring bacterial growth and activity in both marine and freshwater environments have severe limitations. These include a lack of specificity, low signal/background ratio, interference from sediment particles, and sample handling artifacts. This project was designed to provide working field methods for measuring growth rates using molecular biology techniques which address these limitations. Specifically, 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.

APPROACH: 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.

PROJECT TITLE: 91-03 (continued)

We are utilizing standard 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 correlate 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, archaeobacteria, 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. This provides an extremely useful indicator of population response to environmental change.

PROGRESS AND RESULTS: Our primary activities during the first year of this project have been:

- adapting photometer equipment for measuring rRNA-specific fluorescence,
- obtaining and selecting marine bacterial strains with a range of characteristic growth rates,
- optimizing and oligonucleotide hybridization procedures,
- testing procedures for extracting and purifying RNA and DNA from sediment,
- developing a working dual-label method for measuring single-cell fluorescence,
- calibrating fluorescence against measured cellular RNA and DNA concentrations,
- developing a modified thymidine method for bacterial growth rate estimates (used as a parallel measurement system), and

PROJECT TITLE: 91-03 (continued)

- conducting batch-culture and chemostat experiments to relate rRNA-specific fluorescence to independent measures of growth rate.

We measure fluorescence of individual, probe-hybridized cells with a scanning microphotometer (Optica Technology Devices, Inc.) mounted on a Nikon Optiphot microscope. Because cells are examined individually, relatively active and inactive cells can be distinguished. Typical frequency distributions of rRNA-specific fluorescence shift to lower rRNA content at slower growth rates, as for during in the shift from late-exponential to early-stationary phases.

The available data from our laboratory experiments indicate that rRNA content is very highly correlated to marine bacterial growth. In batch culture, rRNA content was highly correlated with cell-specific thymidine incorporation (a method for estimating specific growth rate) for three of four bacterial strains used ($r = 0.78$, $p < 0.001$, $n = 20$). The correlation was extremely strong during exponential growth ($r = 0.99$, $n = 7$). Detailed analysis suggests that much of the variability was due to inherent characteristics of batch cultures; i.e. it is difficult to estimate instantaneous growth rates accurately. We turned to chemostat culture in order to control growth rates precisely. Available chemostat data suggest a virtually perfect correction ($r = 0.99$ and $r = 0.94$ in two experiments) between rRNA content and growth rate. This has been confirmed for biomass doubling times as long as 3 days, comparable to growth rates in the majority of coastal marine systems.

In the summer 1991, we began testing the method in natural samples collected in eutrophic (Georges Bank and Great South Bay/Long Island) and oligotrophic (Sargasso Sea) waters, and are now working on signal enhancements to improve rRNA/fluorescence measurements. The signal enhancement is accomplished by using multiple independent oligonucleotide probes with approximately the same level of taxonomic specificity. We are working to determine whether the fluorescence signal from multiple probes is additive. Finally, we initiated experiments with marine protists which explore the relationship between their RNA content and growth rate in natural seawater culture.

SPECIFIC ACCOMPLISHMENTS: Portions of the chemostat and bath-culture data will be presented at the American Society of Limnology and Oceanography annual meeting in February 1992, and at the 20th Marine Benthic Ecology Meetings in March 1992. A manuscript dealing with these data is in preparation.

PROJECT TITLE: **91-03 (continued)**

PROPOSALS:

1. An invited pre-proposal to the U.S. Environmental Protection Agency was submitted in November 1991.
2. This data also formed the basis of a proposal to the DOE-funded Ocean Margins Program, now under consideration.

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
	1991	\$ 52,763
	1992	<u>56,000</u>
	TOTAL	\$108,763

PROJECT DESCRIPTION:

We have 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 1991:

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 test the hypothesis that a highly specific proteinase whose activity is required for invasion of red blood cells by *P.falciparum*, the organism that causes human malaria, is a legitimate target for rationally designed drugs. The hypothesis will be tested by synthesizing equally specific proteinase inhibitors that should inhibit invasion but not normal physiological processes.

Long term objectives include the development of second generation proteinase inhibitors and attempts to develop an antimalarial vaccine against a recombinant proteinase or fragments of it, either surface sequences or sequences of the active site. If successful, the technology we devise can easily be extended to the treatment of other parasitic diseases.

APPROACH: Our approach to developing a new antimalarial agent is different from those currently being tried. We are not characterizing all the proteinases of merozoites, nor screening a

PROJECT NUMBER: 91-04 (continued)

new series of chemicals for antimalarial activity, nor screening cell surface proteins for an antigen for a vaccine. Although numerous parasite proteinases have been discovered, their functions are, in most cases, unknown. We shall purify a specific proteinase based on its physiological function, invasion of red blood cells. Drugs are being developed against intracellular targets, e.g. derivatives of chloroquine, and vaccines are being developed against surface proteins of unknown function. Our target is an enzyme activity on the surface of the parasite that is directly accessible to agents in the external milieu between merozoite release and reinvasion. As such, if multiple-drug resistance via P-glycoprotein operates in *Plasmodium*, that will have no effect on an agent that acts on the cell surface. Although the sequences of cell surface proteins are highly variable so as to evade the immune system, it may be much more difficult for the parasite to change the active site of the proteinase to evade a specific inhibitor.

PROGRESS AND RESULTS: With the Rhodamine-based substrate (Ile-Pro-Arg-NH)2-Rhodamine we have observed in situ a proteinase activity on the surface of late schizonts and merozoites of *P.falciparum* and *P.chabaudi*. This proteinase must be unusual and highly specific, as its activity can be assayed in the presence of the numerous proteinase inhibitors in serum. We have also shown that bovine pancreatic trypsin inhibitor inhibits reinvasion of red blood cells by *P.falciparum* and *P.chabaudi*. The data imply that only one proteinase is being inhibited by bovine pancreatic trypsin inhibitor. The substrate and the inhibitor are related in that cleavage of (Ile-Pro-Arg-NH)2-Rhodamine by purified merozoites is inhibited by bovine pancreatic trypsin inhibitor at about the same concentration that bovine pancreatic trypsin inhibitor inhibits reinvasion. Thus, we may have a simple assay and an inhibitor for a proteinase activity that is required for reinvasion.

Based upon its affinity for bovine pancreatic trypsin inhibitor, 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 bovine pancreatic trypsin inhibitor. The resultant mutant bovine pancreatic trypsin inhibitor should be a highly specific inhibitor of this proteinase and no others. There are good experimental systems within which to measure the efficacy of bovine pancreatic trypsin inhibitor, from in vitro studies on the inhibition of the recombinant proteinase, to curing a culture of red blood cells infected by *P.falciparum*, to curing a mouse infected by *P.chabaudi*.

PROJECT NUMBER: 91-04 (continued)

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, in press.

PROJECT NUMBER: 91-07

PROJECT TITLE: BASIC STUDIES ON THORON AND THORON PRECURSORS

PRINCIPAL INVESTIGATOR: Harbottle, Garman

LDRD FUNDING:	FISCAL YEAR	AMOUNT
	1991	\$37,941
	1992	<u>52,000</u>
	TOTAL	\$89,941

PROJECT DESCRIPTION:

This project is concerned with the behavior of thoron and its precursors in the thorium-232 chain in soils. Although thoron (Rn-220) is recognized as a health concern, experimental studies are very few compared to those involving radon (Rn-222). This despite the fact that, 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 of radon in soils also necessarily apply to thoron.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1991:

APPROACH: The project utilizes an experimental approach that is modelled on that already in use for radon (Rn222, half-life 3.8 days). Members of the thorium 232 chain are measured through their characteristic gamma ray emission, or that of their short-lived daughter activities. For example, Ra228 is determined through the radiations of its progeny Ac228 (6.15 hours) while Ra224, the precursor of thoron, is estimated by its own gamma radiation and that of its progeny Pb212. Thorium 232 is determined by neutron activation analysis.

The overall radiochemical scheme also permits determination of the fraction of thoron emanation atoms that escape the soil grains and enter the soil gas component. Finally, the measurements permit the determination of departures from secular equilibrium, which are related to processes of soil weathering and radioisotope transport.

PROGRESS AND RESULTS: The thorium-chain radioactivity has been measured in soil samples taken from three soil profiles, deriving from granite weathering, on the slopes of Mt. Major, New Hampshire. These profiles were chosen for two reasons. First, these granite soils are very rich in thorium, and second, the pedological soil structure and evolution is already being investigated by a collaborator, Professor Christine Evans of the Geology Department,

PROJECT NUMBER: 91-07 (continued)

University of New Hampshire, Durham. The progress lies in the successful completion of not only the conventional analyses but also the radiochemical analyses. Work is now proceeding on soil fractions which will reflect the processes of weathering, and permit us to compare thoron and radon escape mechanisms.

SPECIFIC ACCOMPLISHMENTS: An internal, informal report was submitted to a DOE research group at Fort Collins, Colorado (University of Colorado, Dr. Tom Borak): W.-J. Lin and G. Harbottle, Effect of Moisture Content of Soil on Radon and Thoron Emanation Coefficients, Internal Radon Project Report, January 10, 1991. A new apparatus which should greatly improve the sensitivity of determination of radioactive isotopes in soil or other environmental materials is being placed in operation: it is an anti-Compton gamma spectrometer, and consists of an intrinsic Germanium well counter whose pulses are in anti-coincidence with a large (12" x 12") sodium iodide scintillation counter that surrounds it. The advantages are, greater efficiency, greater peak to noise ratio, smaller sample size and reduced geometric and thickness corrections. The use of this device was obtained at no cost to the Thoron project. Preliminary measurements with soils suggest that this device will be of great value in the study of both natural and man-made radioisotopes in environmental samples.

PAPERS/JOURNALS/PUBLICATIONS:

1. W.-J. Lin and G. Harbottle "Gamma Ray Emission Intensities of the ^{232}Th Chain in Secular Equilibrium," J. Radioanalytical Nucl. Chem (in press).
2. W.-J. Lin and G. Harbottle "Determination of Diffusion Lengths and Flux Densities of Radon and Thoron in Soils" Health Physics (submitted).

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, Carl W.

LDRD FUNDING:	FISCAL YEAR	AMOUNT
	1991	\$ 85,318
	1992	<u>90,000</u>
	TOTAL	\$175,318

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 the dsDNA-kinase.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1991:

The 350,000 dalton 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 ~2000 base-pair (bp) cDNA insert; a second clone containing an overlapping ~2000 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 kb in length.

Sequence from the first clone was subcloned into an E. coli expression vector and the resulting fusion protein was purified and

PROJECT NUMBER: 91-08 (continued)

used to immunize a rabbit. Hyperimmune serum from the rabbit reacted with a 350kD polypeptide in crude lysates of HeLa cells and with the 350-kD polypeptide in a purified kinase preparation. These results strongly suggest that the clones obtained correspond to the cDNA for DNA-PK. Efforts to obtain additional cDNA clones corresponding to remaining regions of the DNA-PK cDNA and to map the position of the DNA-PK gene by hybridization in situ to spreads of human chromosomes are in progress.

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.

Analysis of DNA-PK activation using human adenovirus DNA suggests that activation may require DNA ends; thus, 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. These new findings suggest that, in addition to its role in transcription, DNA-PK may monitor DNA for damage and coordinate the cellular response to chromatin damage.

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).

PROJECT NUMBER: 91-09

PROJECT TITLE: COMBINED BORON NEUTRON CAPTURE THERAPY (BNCT)
AND ADOPTIVE IMMUNOTHERAPY IN THE TREATMENT OF
EXPERIMENTAL BRAIN TUMORS

PRINCIPAL INVESTIGATOR: Joel, Darrel D.

LDRD FUNDING:	FISCAL YEAR	AMOUNT
	1991	\$34,869
	TOTAL	\$34,869

PROJECT DESCRIPTION:

Although BNCT has been shown to be an effective radiotherapeutic modality for intracranial gliomas in rats, it is likely that better results will be obtained in humans when BNCT is combined with other treatments. Preliminary results suggest that BNCT enhances lymphocytic infiltration into the treated tumor. Successful adoptive immunotherapy, in turn, requires that a sufficient number of transferred effector lymphocytes extravasate at sites of tumor growth to mediate their antitumor function. Thus, the principal objective of this proposal is to devise a protocol for the BNCT-induced enhancement of immunological mediated tumor inhibition in vivo using our defined rat brain tumor model.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1991:

The tumor model used in these studies is a transplantable gliosarcoma (brain tumor) which was originally induced in rats (Fisher 344) by injections of N-nitrosomethylurea. The cell line, which is easily maintained in tissue culture, was brought to BNL and developed experimentally with support from a previous LDRD. This work subsequently lead to a DOE-funded FWP entitled: evaluation of Boron Ccompounds for Neutron Capture Therapy [MO-18 (004784)].

It appears likely that even with optimal boron neutron capture therapy (BNCT) better results will be obtained when BNCT is combined with other treatment modalities such as adoptive immunotherapy with lymphokine-activated killer (LAK) cells or tumor infiltrating lymphocytes (TIL). LAK cells were generated from lymphocytes isolated from spleens of normal or tumor immunized rats. Spleens were minced and the resulting cells suspensions passed over Ficoll-Hypaque density gradients and nylon-wool columns yielding enriched lymphocyte suspensions. Lymphocyte suspensions were then cultured for several days in the presence of media containing interleukin-2 (IL-2). LAK cells generated by this method, were capable of killing tumor cells in vitro which was demonstrated by the release of ⁵¹Cr from labeled gliosarcoma cells.

PROJECT NUMBER: 91-09 (continued)

LAK cells kill tumor indiscriminately i.e. they do not respond to specific tumor cell surface antigens.

In contrast, activated killer cells generated from tumor infiltrating lymphocytes (TIL) are highly specific for the tumor type from which they were isolated. TIL were generated from actively growing gliosarcomas surgically removed from rats on the tenth day after initiation by the subcutaneous injection of cultured tumor cells. Tumors were minced and the resulting cell suspensions were cultured with IL-2. With time, lymphoid cell numbers increased and tumor cells decreased until all that remained were lymphocytes. When analyzed for the capacity to kill gliosarcoma cells in vitro, TIL were more effective, on a per cell basis, than LAK cells.

The principal objective of this proposal is to determine whether BNCT would augment tumor uptake of circulating LAK cells or TIL, thereby enhancing tumor control. BNCT experiments are best interpreted when compared to results obtained with equivalent doses of photon irradiation. Our initial studies were thus done using a 250 kVp x-ray generator.

A subcutaneous tumor was initiated on each hind leg of rats by the injection of 5×10^6 cultured gliosarcoma cells. Ten days later, when the tumors were between 0.4 and 1.0 grams, one leg tumor was exposed to 22.5 Gy x-rays. The tumor on the opposite leg, as well as the rest of the body, was lead shielded during radiation exposure. One day later and each rat was injected intravenously with ^{51}Cr -labeled LAK cells. Twenty-four hours after the injection of LAK cells the rats were euthanized and radioactivity of each of the hind leg tumors measured in a gamma scintillation counter and expressed as cpm/gram tumor. In all 7 animals the radioactivity (LAK cell infiltration) was greater in the irradiated tumor than in the shielded tumor. On the average there was 50 percent increase in radioactivity in irradiated tumors.

PROJECT NUMBER: 91-10

PROJECT TITLE: FEASIBILITY STUDY FOR MICROBEAM RADIATION THERAPY WITH 30-90 keV X-RAYS FROM THE NSLS X17 BEAMLINE

PRINCIPAL INVESTIGATOR: Slatkin, Daniel N.

LDRD FUNDING:	FISCAL YEAR	AMOUNT
	1991	<u>\$60,742</u>
	TOTAL	\$60,742

PROJECT DESCRIPTION:

This LDRD proposes to carry out feasibility studies in the mouse for the use of monochromatic x-rays from the X17 superconducting wiggler at the NSLS for Microbeam Radiation Therapy (MRT). The studies to be conducted under this LDRD will be: a) Design and assemble a set of computer-driven beam slits to provide square microbeams of 20-1000 micrometer dimensions; b) Design and assemble a set of positioning stands for the animal apparatus to position the animal in the beam and to move it across the beam in steps of 50-100 micrometers; c) Carry out experiments in which the mouse cerebrum, cerebellum, and eye are irradiated unilaterally with a single monochromatic microbeam at energies of about 30, 60, and 90 keV, at beam dimensions of 25, 50, 100, and 1000 micrometers, and with doses of 20,000, 100,000, and 400,000 rad; d) Evaluate mouse neuropathology and ophthalmic pathology as a function of these irradiation parameters; and e) Repeat the studies indicated in sections (c) and (d) for selected energies, beam dimensions, and radiation doses, with a square pattern of 25 equally spaced parallel pencil beams with a center-to-center beam spacing in the 50- to 150-micrometer range. The goal of the latter study will be to discover the critical interbeam separation at which the probability for delayed CNS radiation necrosis is expected to increase sharply with decreasing separation.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1991:

Synchrotron radiation from the wiggler insertion device of the X17B beamline at the National Synchrotron Light Source (NSLS) is practically non-divergent and has enormous flux at photon energies under 100 keV. This means that it is suitable for development of a new type of radiosurgery called 'microbeam radiation therapy' (MRT). As was recently predicted from studies by Curtis et al. using 22 MeV deuteron microbeams at Brookhaven National Laboratory (BNL) three decades ago, and from recent Monte Carlo photon transport and secondary-electron transport computations, a spatially fractionated bundle of parallel, 25 μm -wide, 4 mm-high,

PROJECT NUMBER: 91-10 (continued)

40-55 keV microbeams (delivered at ≈ 250 gray per second) yields no cerebrovascular damage in the rat one month after irradiation, even at peak microbeam doses of 2500 or 5000 gray. A 1 mm x 1 mm pencil beam of the same quality of radiation delivered at the same dose rate without such spatial fractionation produces colliquative necrosis of the rat cerebrum in its path within two weeks after a dose of only 156 gray.

It is predicted that multiple crossfired bundles of appropriately-dimensioned parallel, planar, synchrotron x-ray microbeams in the 50-150 keV range will ablate tissues in the target zone of a human brain where the bundles overlap, but will spare tissues where they do not overlap when peak/valley doses are as much as 100 gray/5 gray. Such MRT will help to renew radiotherapy in pediatric neuro-oncology and will treat tumors in patients of any age with greatly diminished probability of collateral damage to non-targeted CNS vital structures such as medulla and spinal cord.

MICROBEAM RADIATION THERAPY: Microscopically fractionated 40-55 keV synchrotron x-rays produce no cerebrovascular damage in the rat at doses up to 5000 gray. For MRT, this means that crossfired bundles of planar microbeams will ablate targeted CNS tissues and spare non-targeted tissues when a peak microbeam dose in the ~ 10 -100 gray range is delivered in several milliseconds.

PROJECT NUMBER: 91-11

PROJECT TITLE: PROPOSAL FOR DEVELOPMENT OF DEPOSITION METHODS FOR MICROMECHANICS

LDRD FUNDING:	FISCAL YEAR	AMOUNT
	1991	<u>\$76,483</u>
	TOTAL	\$76,483

PRINCIPAL INVESTIGATOR: Warren, John B.

PROJECT DESCRIPTION:

It is proposed that the LIGA process (a synchrotron-based lithography method used for the fabrication of microcomponents with vertical aspect ratios of more than 100:1) be adapted to advanced deposition methods such as chemical vapor deposition and plasma enhanced chemical vapor deposition. If successful, these deposition techniques will allow the formation of polycrystalline microstructural components from tungsten and diamond, greatly expanding the range of materials used for the construction of devices in the emerging field of micromechanics.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1991:

PURPOSE: The objective of this project was to develop plasma enhanced deposition methods for the fabrication of microstructures useful for micromechanics and materials testing. A sacrificial microstructure is first created in an PMMA x-ray photoresist layer by using highly collimated radiation from the U2A x-ray lithography beam line at the NSLS. The sacrificial resist can be as much as several hundred microns thick. Plasma deposition is then used to coat the resist microstructure. After deposition, the resist is removed, leaving the completed microcomponent intact. Attempts were made to form test microstructures from high strength and high elastic modulus materials such as tungsten and diamond.

APPROACH: Specific lithography and deposition methods were developed to produce the microstructures. A new hot stage was ordered and installed in the Plasma Technology plasma enhanced deposition reactor in the Instrumentation Division to allow diamond deposition at temperatures up to 1000 degrees celsius. Work was begun with N. DeLanerolle (Standard Microsystems, Hauppauge, NY) on the characterization of sputtered tungsten thin films. With the assistance of Ron DellaGuardia (IBM), several exposure series of thick PMMA x-ray photoresist layers were completed at the U2A x-ray lithography beam line. X-ray masks with the patterns required to produce the microstructures were fabricated and used for exposure sequences at U2A. The mask patterns were accurately replicated in

PROJECT NUMBER: 91-11 (continued)

PMMA resist layers 7-8 microns in thickness. On March 15, 1991 formal approval was granted by the NSLS General User Review Committee for a total of 14 days further experimentation on the U2A beam line during the May - Aug. 1991 experimental period.

PROGRESS AND RESULTS: The most important result to date is the development of an integrated mask/wafer assembly that eliminates the requirement of a costly x-ray mask. In normal practice, the x-ray mask consists of gold absorbing regions on a silicon membrane 1-2 microns in thickness. The membrane is mounted on a pyrex ring and positioned approximately 50 microns from the PMMA-coated wafer during exposure. This type of x-ray mask is fragile and costly to fabricate. By patterning a deposited absorbing layer directly on the PMMA resist with e-beam or optical lithography, the requirement for a separate mask is eliminated. This new technique greatly increases the number of microstructures that can be fabricated after a typical exposure series.

PAPERS/JOURNALS/PUBLICATIONS:

1. Materials Characterization for Micromechanics Using Deep Etch Lithography, J. B. Warren, Proc. of Active Materials and Adaptive Structures, Nov. 4-8, 1991, Arlington, VA. (in press)

PROPOSALS:

1. Micromachined Structures for Applications in Far Infrared Filters, W. Carr, D. Moeller (New Jersey Institute of Technology), J.B. Warren, D.P. Siddons (Brookhaven National Laboratory). To be submitted to the National Science Foundation.

PROJECT NUMBER: 91-15

PROJECT TITLE: PHOTON DATA AUTOMATION

PRINCIPAL INVESTIGATOR: Dunford, Charles

LDRD FUNDING:	FISCAL YEAR	AMOUNT
	1991	<u>\$29,076</u>
	TOTAL	\$29,076

PROJECT DESCRIPTION:

A computerized system of data storage and retrieval will be developed appropriate to synchrotron radiation physics. A six-month pilot program will be conducted that will place selected data measured at NSLS beam lines into the system and provide worldwide network access to the information by users at BNL and other facilities.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1991:

PURPOSE: This project was designed to investigate the feasibility of establishing a data center for the compilation and dissemination of experimental and recommended x-ray absorption fine spectra data.

APPROACH: The National Nuclear Data Center has more than 30 years experience in the compilation, evaluation and dissemination of nuclear physics data. The center has a VAX computer with worldwide access to computer networks. The strategy was to take advantage of both the computer facilities and the data compilation and dissemination experience to extend existing capability to a new data field where such a capability had been requested by researchers. In particular, the object was to demonstrate that the formats and procedures developed for nuclear physics data could easily be extended to a new area of physics and then provided to researchers over computer networks via the existing NNDC on-line data service.

RESULTS: The following tasks have been completed:

1. The nuclear physics data storage format, EXFOR, had been successfully extended to this new data type.
2. Existing storage and retrieval programs had been modified to handle the extended format.
3. Several sets of experimental data from the NSLS at BNL were compiled and stored in a data base.

PROJECT NUMBER: 91-15 (continued)

4. A data retrieval program was developed and successfully tested in the framework of the on-line data service.
5. A data base of photon interaction cross sections and attenuation coefficients and polarized photon-beam scattering cross was developed and included in the on-line data service.

The project successfully reached all of its goals. Unfortunately, no permanent funding was found to establish the planned data center.

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
	1991	\$ 79,657
	1992	<u>100,000</u>
	TOTAL	\$179,657

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 ($>5\text{kHz}$), complete tunability from 1000 nm to 300 nm, high peak energy ($> 25\mu\text{j}$) 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 1991:

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

APPROACH: The prototype photocathode/seed laser system uses a regenerative amplification scheme to directly amplify weak, broadly tunable, picosecond pulses from more conventional synchronously-pumped dye lasers. High repetition regenerative amplification schemes have been developed in the Chemistry Department for fixed 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 $25\mu\text{j}$. Our current system in the Chemistry Department can produce gigawatt peak powers at repetition rates up to 3 KHz and rates as high as 10 KHz should be possible. The utilization of all solid state components also ensures maximum reliability and low maintenance costs.

PROJECT NUMBER: 91-18 (continued)

TECHNICAL PROGRESS AND RESULTS: We have successfully completed the implementation of the high repetition rate "dye-laser" consisting of a mode-locked Ti-Sapphire laser pumped by a cw, ion laser. The Ti-Sapphire laser can produce tunable radiation from 780 nm to 1100 nm with ultrafast pulse lengths approaching 200 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 output characteristics of the Ti-Sapphire laser were thoroughly characterized and found to meet our requirements in terms of pulse width, pulse energy, beam quality and beam stability. These studies 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. In addition, initial design and fabrication of the regenerative amplifier section was begun and this work will continue into FY 92.

EXPECTED PROGRESS FY 92: The final phase of the regenerative amplifier fabrication will be completed and characterization of the optical properties of the resonator will be performed. The Ti-Sapphire regenerative amplifier is unique in that it eliminates the need for an additional dye-based amplification stage typical of all current laser systems. This work should lead to our goal of producing tunable laser light (1000 nm - 300 nm) with ultrashort pulse lengths (200 fs - 6 ps), high peak powers (>1 GW) and high repetition rate (~5 kHz). The laser technology developed over the project term will then be transferred to the NSLS/ATF laser staff who will begin implementation of a second generation Ti-Sapphire laser system for collaborative photocathode studies.

PROJECT NUMBER: 91-19

PROJECT TITLE: CLUSTER IMPACT FUSION

PRINCIPAL INVESTIGATOR: Friedman, L.

LDRD FUNDING:	FISCAL YEAR	AMOUNT
	1991	\$29,696
	1992	<u>50,000</u>
	TOTAL	\$79,696

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 1991:

Impacts of accelerated cluster ions containing hundreds of atoms onto surfaces have been shown to be an important research tool for the study of high transient pressures and high energy density thermal spikes. An important feature of these spikes is that the conditions are approaching, on a microscopic scale, those required for very high temperature phenomena, including thermonuclear fusion, to take place. Up until the present time, the accelerated cluster ions have had only a single charge. The only way to increase the energy had been to increase the acceleration voltage, and a practical limit of approximately 600,000 volts has been achieved at the Cockcroft Walton accelerator located at the Brookhaven Linac. Efforts to increase this voltage do not appear to be cost effective at the present time.

An alternative approach to increasing the energy of the cluster is to increase the charge state of the ion. One practical source of multicharged ions is the electrospray process where a solution is sprayed from a highly charged hypodermic needle. The process is very complex, involving both the fragmentation and evaporation of the highly charged droplets. This source was delivered in late FY 1991 and is presently being installed in a vacuum system especially fabricated to accept this source. The output of the source will be mass analyzed in a unique high molecular weight mass spectrometer and then accelerated into a sensitive ion detector. Both the mass spectrometer and ion detector were fabricated and tested in FY 1991 in preparation for the multicharged ion source.

PROJECT NUMBER: 91-20

PROJECT TITLE: A SEQUENCE RELATED TO THE HUMAN GONADOLIBERIN
PRECURSOR NEAR THE N-TERMINI OF HIV AND SIV
GAG POLYPROTEINS (GONADOLIBERIN PRECURSOR
MIMICRY IN HIV P-17: AIDS VACCINE AND
ANTIVIRAL PEPTIDES)

PRINCIPAL INVESTIGATOR: Glass, J.D.

LDRD FUNDING:	FISCAL YEAR	AMOUNT
	1991	\$54,998
	TOTAL	\$54,998

PROJECT DESCRIPTION:

In preliminary studies, it was found that all strains of the AIDS virus (HIV) and all strains of related viruses that cause AIDS-like diseases in monkeys (SIV) have a peptide sequence in one of their proteins that is closely related to a precursor protein for the human hormone, gonadoliberein. One can tell from the structure of this viral peptide sequence that it requires processing by the same types of enzymes that release the human hormone from its precursor protein and we can tell that this enzymatic processing probably occurs while the virus is outside of human cells. Furthermore, the hormone-precursor-related viral peptide sequence remains quite constant in viral strains that are rapidly mutating. Taken together, these findings suggest that one has an unusually good chance to make synthetic drugs and synthetic vaccines that will protect against infection with AIDS and, perhaps, control the progress of the disease in persons already infected.

VACCINE - What we need to know is 1) whether one can raise antibodies against this peptide sequence and 2) whether antibodies against this peptide will neutralize the virus. The HPG-30 peptide is now in trials as a possible vaccine against AIDS but the homology with thymosin alpha-1 and the degree of sequence conservation from viral strain to viral strain is such that the vaccine is likely to be outflanked by mutations. We want to synthesize Leu-Arg-Gly-Pro-Gly-Gly-Lys-Lys-NH₂ and two other peptide sequences corresponding to intermediates in processing of the HIV processing site, to couple these to carrier proteins, and to immunize rabbits against the peptides. These antisera will be tested at Stony Brook to see if they block replication of HIV in cultured cells. If they do we will have the best candidate for a stable vaccine against AIDS that we are aware of. We also want to synthesize the peptide Arg-Phe-Gly-Lys-Lys-NH₂ and raise antisera against it in rabbits. These antisera will be tested by Plum Island Animal Disease Laboratory for their ability to block infectivity of bluetongue virus in vitro. If this works, we will have a good animal model to use in development of a vaccine against

PROJECT NUMBER: 91-20 (continued)

AIDS. This is important because HIV infects only humans and chimpanzees, both in short supply as laboratory animals.

INHIBITORS OF A PROTEASE PRESUMED TO BE INVOLVED IN PROCESSING OF THE VIRAL AMIDATION SITE - For a variety of reasons we think that the enzymatic reactions involved in processing the gonadoliberein-precursor-related sequence in HIV probably occur outside of the cell - exposed to drugs in the general circulation. We are outside of the cell - exposed to drugs in the general circulation. We are particularly interested in designing peptide inhibitors of the calcium-dependent serine protease that catalyzes the first step in the processing of amidation sites such as that in the viral p-17 core protein. We propose to use methods (worked out by Dr. E. Shaw) to make variations on the peptide derivatives Leu-Arg-Pro-Gly-Lys-Lys-CH₂Cl and Arg-Phe-Gly-Lys-Lys-CH₂Cl which will be tested for their ability to block infectivity of HIV and bluetongue virus, respectively, in cell cultures.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1991:

In fiscal year 1991, theoretical studies leading to the discovery of the gonadoliberein precursor mimic sequence in HIV and SIV p-17 core proteins was published and a second of the three conserved "amidation sites" in HIV-1 proteins (in gp-41 transmembrane protein) was correlated with a specific post-translational processing site in another human hormone precursor protein (SP2 amidation site in proglucagon). A manuscript describing the identification of this second mimic sequence is now in review.

The peptide sequence Leu-Arg-Pro-Gly-Gly-Lys-NH₂ (conserved gonadoliberein precursor mimic sequence) was synthesized and conjugated to keyhole limpet hemocyanin. A rabbit was immunized with this peptide-protein conjugate. Resulting antisera recognized the free peptide in an ELISA immunoassay. These antisera were lost in a freezer failure, but reserve samples of synthetic peptide and peptide-protein conjugate are available for a repeat immunization.

An assay to test inhibition of HIV replication in human macrophage and lymphocyte cultures was established in collaborative studies with Dr. Sidonie Morrison of the Hematology Department at SUNY-Stony Brook. Preliminary studies in this assay suggest that gonadoliberein (LHRH) inhibits viral infectivity or replication in this assay system, as predicted by our hypothesis that HIV uses the gonadoliberein mimic sequence for interaction with the gonadoliberein receptor. Studies to confirm and extend these preliminary results are in progress.

PROJECT NUMBER: 91-20 (continued)

A peptide corresponding to the proglucagon mimic sequence in gp-41 has also been synthesized. This will also be used to test for inhibition of viral replication of HIV in cultured cells, again in collaboration with Dr. Morrison.

An abstract describing discovery and analysis of the two hormone-precursor-mimic sequences in HIV proteins has been accepted for presentation at the Keystone Conference on AIDS, to be held at Keystone, Colorado in the week overlapping the end of March and the beginning of April, 1992.

The article in J. Theor. Biol. was cited by "Human Retroviruses and AIDS" (a periodic release from the HIV sequence database maintained by the Theoretical Biology group at Los Alamos National Laboratory) within weeks of its publication along with a notation that this conserved hormone precursor mimic sequence overlaps a reported cellular immunity epitope.

Application for continuation support from NIH was reviewed as "a novel and imaginative approach to a difficult and important problem ...while new and provocative ideas for inhibiting the replication and spread of HIV are in short supply". The proposal was deemed premature, however, and lacking in preliminary evidence. It is hoped that the results now coming out of the collaborative studies with Dr. Morrison will satisfy this deficiency, and another proposal to NIH is planned once these results are replicated.

PAPERS/JOURNALS/PUBLICATIONS:

1. Glass, John D. "A Sequence Related to the Human Gonadoliberein Precursor Near the N-termini of HIV and SIV gag Polyproteins (1991) Journal of Theoretical Biology 150, 489-496.
2. Glass, John D. "A Proglucagon-Related Sequence in HIV-1 gp-41 Transmembrane Protein, Submitted to J. Theor. Biol.

PROJECT NUMBER: 91-21

PROJECT TITLE: EFFECT OF A BACTERIAL SPORE PROTEIN ON
MUTAGENESIS

PRINCIPAL INVESTIGATOR: Setlow, Jane K.

LDRD FUNDING:	FISCAL YEAR	AMOUNT
	1991	\$ 87,461
	1992	<u>160,000</u>
	TOTAL	\$247,461

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 1991:

The most important result is the discovery that DNA conformation is a significant factor influencing spontaneous mutation. When a gene from the spore-forming bacterium Bacillus Subtilis is induced to make a protein which increases supercoiling of DNA in Escherichia coli, the spontaneous mutation is considerably increased. The B. subtilis gene is in a circular piece of DNA called a plasmid which replicates independently from the chromosome of the E. coli cell. A control experiment with a slightly altered B. subtilis gene that produces a protein made in similar amounts to the wild-type protein but fails to bind a DNA and does not cause mutation shows conclusively that binding to DNA by the protein is required for the increase in mutation.

The mutations caused by the B. subtilis protein are single-base changes in the DNA, mostly from A - T base pairs into G - C pairs (transitions), rather than into T - A or C - G pairs (transversions).

A number of mutations in E. coli have been found to influence the effect of the B. subtilis protein. A defect in the E. coli recA gene abolished the B. subtilis protein's effect on mutation. Defective umuC and umuD genes caused elimination of the most of the mutation caused by that protein, whereas loss of the lexA gene caused increased mutation. These data suggested that the observed increased mutation in the presence of the protein was a response to DNA damage caused by conformation change, inducing a type of repair known to be error-prone. However, if this were so, then the RecA

PROJECT NUMBER: 91-21 (continued)

protein should be made in large amounts when the B. subtilis protein is induced, which we have found not to be the case. Thus the molecular mechanism of the mutagenic effect of DNA conformation remains to be deciphered.

PAPERS/JOURNALS/PUBLICATIONS:

1. Submitted for publication to the Journal of Bacteriology: Mutation and killing of Escherichia coli expressing a cloned Bacillus subtilis gene whose produce alters DNA conformation.

PROJECT NUMBER: 91-22

PROJECT TITLE: DEVELOPMENT OF SUPERFERRIC MICRO-UNDULATORS

PRINCIPAL INVESTIGATOR: Ben-Zvi, Ilan

LDRD FUNDING:	FISCAL YEAR	AMOUNT
	1991	<u>\$100,001</u>
	TOTAL	\$100,001

PROJECT DESCRIPTION:

The purpose of this project is to develop electromagnet undulators with sub centimeter periods capable of serving as an insertion device in synchrotron light sources and Free-Electron Laser for the generation of intense, short wave-length radiation. The micro-undulator will be based on a superferric electromagnet, using a superconducting coils wound in alternating directions on a ferromagnetic yoke. The micro-undulator will have a short period of 0.88 cm with a large gap of 0.44 cm, however will provide a high peak axial of about 0.5 Tesla. A full sized micro-undulator (about 70 periods) will be constructed and used to operate a visible Free-Electron Laser at the BNL Accelerator Test Facility.

TECHNICAL PROGRESS AND RESULTS - FISCAL YEAR 1991:

PURPOSE: This project focussed on the development, fabrication and testing of a superconducting electromagnet undulator with a very small period, 8.8 mm. The advantages of such an undulator are a combination of a high magnetic field and very short period, suitable for the generation of very short, intense radiation from synchrotron storage rings and Free-Electron Lasers.

With the advent of laser photocathode rf electron guns, linear accelerators are able to produce high current, low emittance beams which are applicable to the construction of VUV and even XUV Free Electron Lasers. The spectral brightness of these FELs will be many orders of magnitude larger than insertion devices on the largest synchrotron light sources and will provide new scientific opportunities in a number of sciences. One example is state-resolved studies of the dynamics of small molecular systems through FEL induced photo-dissociation.

APPROACH: The BNL Accelerator Test Facility comprises a unique combination of a high brightness 50 MeV linac and a high power short pulse laser complex synchronized to the linac. It incorporates a novel 2856 MHz laser photocathode rf gun which provides high current, short pulses (≤ 10 ps time width) with a small emittance due to high cathode rf electric fields (100 MV/m).

PROJECT NUMBER: 91-22 (continued)

These characteristics make the ATF a most suitable facility for short wavelength Free Electron, and an ideal test bed for novel short wavelength undulators.

The Visible FEL experiment aims at demonstrating the capabilities of a laser photocathode rf gun of the BNL design for the generation of intense, coherent FEL radiation at short wavelength from a low energy electron beam. The ATF electron beam energy initially will be 50 MeV and we expect to demonstrate visible (5000Å) FEL operation with this beam. With a subsequent increase of the beam energy and improvement in the electron gun emittance we expect to reduce the FEL wavelength to the UV. The Visible FEL will be used as a test bed and demonstration for FEL techniques and concepts ultimately leading to high power, short wavelength FELs for a User Facility at BNL.

RESULTS: The test results of a section of the superconducting micro-undulator are reported in the paper by I. Ben-Zvi, R. Fernor, J. Gallardo, G. Ingold, W. Sampson, M. Woodle, Performance of a Superferric, High Field Sub-centimeter Undulator, Proceedings of the 1991 International Free-Electron Laser Conference, of a UV Free Electron Laser User Facility at the National Synchrotron Santa Fe NM.

The bottom line of this paper, is that the undulator has performed even better than expected, providing a magnetic field on axis of more than 0.5 Tesla. This field is much better than can be achieved by any conventional undulator at this gap (4.4 mm) and period (8.8 mm). Furthermore, the field accuracy of the undulator has been surprisingly good, better than 0.3% rms ΔB over B as manufactured, without any adjustment. This result has been limited by the precision of the measurement device and is expected to be better. Thus this undulator turns out to be also quite inexpensive, since the major part of the cost of conventional undulators is in the adjustment sequence.

The success of this undulator has led the NSLS to a major decision, to base the proposed UV-FEL Users' Facility of this undulator technology. Furthermore, a collaboration with Grumman Aerospace Industry has been set up, in which Grumman is building a superconducting undulator based on the BNL development. This undulator will be used on yet another novel experiment at the ATF, the High Gain Harmonic Generation experiment. This experiment, a BNL-Grumman collaboration, will test the basic FEL physics principles of the UV-FEL and its undulator technology.

This program is expected to lead to additional funding for the development of a UV-FEL Users' Facility at the National Synchrotron Light Source. A proposal for this facility is being prepared by the NSLS department and will be submitted to the Basic Energy Science Division of the Department of Energy.

PROJECT NUMBER: 91-22 (continued)

PAPERS/JOURNALS/PUBLICATIONS:

1. I. Ben-Zvi, et al, The Performance of a Superconducting Micro-Undulator Prototype, BNL-44677 1990.
2. I. Ben-Zvi, et al, Proposed UV-FEL User Facility at BNL, 1990 International FEL Conference, Paris. BNL-45161 1990.
3. K. Batchelor, I. Ben-Zvi, R. Fernor, J. Gallardo, H. Kirk, C. Pellegrini, A. van Steenbergen and A. Bhowmik, A Microwiggler Free-Electron Laser at the Brookhaven Accelerator Test Facility, Nucl. Instr. and Meth. A296 239, (1990).
4. I. Ben-Zvi, R. Fernor, J. Gallardo, G. Ingold, W. Sampson, M. Woodle, Theoretical Analysis of Iron-Bore Superconducting Undulators, Proceedings of the 1991 International Free-Electron Laser Conference, Santa Fe NM.
5. K. Batchelor, I. Ben-Zvi, R. C. Fernor, A. S. Fisher, A. Friedman, J. Gallardo, G. Ingold, H. Kirk, S. Kramer, L. Lin, J. T. Rogers, J. F. Sheehan, A. van Steenbergen, M. Woodle, J. Xie, L. H. Yu and R. Zhang, Status of the Visible Free-Electron Laser at the Brookhaven Accelerator Test Facility, Proceedings of the 1991 International Free-Electron Laser Conference, Santa Fe NM.
6. I. Ben-Zvi, L.F. DiMauro, S. Krinsky, M.G. White, L.H. Yu, K. Batchelor, A. Friedman, A.S. Fisher, H. Halama, G. Ingold, E.D. Johnson, S. Kramer, J.T. Rogers, L. Solomon, J. Wachtel and X. Zhang, Proposed UV-FEL User Facility at BNL, Proceedings of the 1991 International Free-Electron Laser Conference, Santa Fe NM.
7. 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, Proceedings of the 1991 International Free-Electron Laser Conference, Santa Fe, NM.
8. I. Ben-Zvi, R. Fernor, J. Gallardo, G. Ingold, W. Sampson, M. Woodle, Performance of a Superferric, High Field Subcentimeter Undulator, Proceedings of the 1991 International Free-Electron Laser Conference, Santa Fe NM.

PROJECT NUMBER: 91-23

**PROJECT TITLE: STRUCTURE AND FUNCTION OF ADENOVIRUS PENTON
BASE PROTEIN**

PRINCIPAL INVESTIGATOR: Freimuth, Paul I.

LDRD FUNDING:	FISCAL YEAR	AMOUNT
	1991	\$ 64,623
	1992	<u>140,000</u>
	TOTAL	\$204,623

PROJECT DESCRIPTION:

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 1991:

PURPOSE: Adenovirus attaches to a specific receptor on the cell surface. A small portion of the cell plasma membrane then engulfs attached virus particles to form intracellular vesicles. Many different non-enveloped viruses are now recognized to escape from similar intracellular vesicles into the cytoplasm to initiate infection, however the mechanism and structural basis for disruption of vesicle membranes by these viruses is not well understood. Based on its detergent-binding properties, the adenovirus penton base subunit is thought to function in membrane lysis. Our genetic and biochemical study of penton base function is expected to provide insights into virus entry mechanisms.

APPROACH AND RESULTS: We have been developing a system to study penton base function by mutagenesis. This system involves the introduction of defined mutations into cloned penton base genes, and the transfer of these mutations into infectious adenovirus genomes for production of virus mutants. Small insertion mutations were constructed at sites in the cloned gene that corresponded to regions of penton base predicted to have detergent-like activity. Each mutation resulted in the insertion of four amino acids into the protein. Similar mutations also were constructed throughout the gene at random sites. To produce virus mutants, the cloned, mutagenized penton base genes were transfected into cultured cells along with overlapping fragments of the adenovirus chromosome. Infectious adenovirus DNA arises in transfected cells by homologous recombination between the overlapping DNA fragments. We have recovered four virus mutants by this procedure. Two of these have insertions in a long hydrophobic segment near the amino terminal end of penton base and appear to have both quantitative and kinetic

PROJECT NUMBER: 91-23 (continue 1)

defects in virus replication. This analysis will continue through the next funding period.

To facilitate biochemical and biophysical analysis, we expressed the penton base gene in bacterial cells using the T7 expression system developed at the BNL Biology Department by Dunn and Studier. Large quantities of penton base are synthesized in this system, however the protein is insoluble and forms a precipitate in bacterial cells. By repeated washing of the insoluble fraction of cell lysates, a highly purified penton base preparation is obtained. The protein precipitate can be dissolved in 8M urea and renatured into a soluble form by sequential dialysis against an alkaline buffer followed by a neutral pH buffer. Using this procedure, milligram quantities of protein can be purified in a single day. The renatured protein contains a toxin-like activity that is also present in the native protein isolated from adenovirus-infected cells. Within one hour of exposure of adherent fibroblast cell cultures to penton base preparations, the well-spread cells round-up and detach from the culture surface. Using deletion analysis we mapped the cell-detaching activity to a 17 amino acid region near the middle of the penton base polypeptide. During the next funding period we will further characterize this ligand, identify the cell receptor it binds to, and assess the role of this interaction in adenovirus replication.

SPECIFIC ACCOMPLISHMENTS: We have submitted a grant proposal to NIH (2/92 deadline) for funding to follow up our initial observations on penton base structure and function.

END

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4 / 13 / 92