Prepared by Deanna Quintana and Donna Spitzmiller, LS-Division

The four most recent reports in this series, unclassified, are LA-10512-PR, LA-10741-PR, LA-11105-PR, and LA-11235-PR.

This work was supported by the U.S. Department of Energy; the National Institutes of Health; the Department of Defense; the U.S. Army Medical Research and Development Command; the U.S. Army Chemical Research, Development, and Engineering Center; the Army Research Institute; the Department of the Interior; the National Aeronautics and Space Administration; and the U.S. Department of Agriculture.

An Affirmative Action/Equal Opportunity Employer

LA-11559-PR
Progress Report

UC-408
Issued: April 1989

LA--11559-PR

DE89 010544

Life Sciences Division

Annual Report 1988

Cover captions

Top series

Calculated sources of visual-evoked neuromagnetic activity are superimposed on magnetic resonance images (MRIs) obtained from the same subject. Circles illustrate the locations of current dipole equivalent sources calculated by model fitting procedures at 10-ms intervals from 90 ms (red) to 150 ms (yellow) following stimulus onset. Crosshairs locate the coronal sagittal, and horizontal planes of MRI slices in the orthographic projections. Individual slices are overlaid on a silhouette produced from an entire series of slices in order to locate the slice with respect to the head volume. During the time sequence illustrated, the source appears to migrate deeper into striate cortex and upward to a location near the parietal-occipital sulcus.

Bottom series

Color contour plot of neutron diffraction data from Tobacco Mosaic Virus particles oriented in magnetized ferrofluid. The darker colors indicate regions where the neutron intensity is greater.

Los Alamos National Laboratory
Los Alamos, New Mexico 87545

CONTENTS

LIFE SCIENCES DIVISION

ANNUAL REPORT

1988

Compiled by

B. L. Marrone, LS-4
L. S. Cram, LS-Division Office

ABSTRACT

This report summarizes the research and development activities of Los Alamos National Laboratory's Life Sciences Division for the calendar year 1988.

Technical reports related to the current status of projects are presented in sufficient detail to permit the informed reader to assess their scope and significance. Summaries useful to the casual reader desiring general information have been prepared by the Group Leaders and appear in each group overview. Investigators on the staff of the Life Sciences Division will be pleased to provide further information.

OVERVIEW

LIFE SCIENCES DIVISION

The missions of the Life Sciences (LS) Division at Los Alamos are to serve the nation's needs in health, energy, and national security by addressing a broad range of biological problems at the molecular, cellular, physiological, and whole-organism levels. LS Division develops and uses the most advanced biological technologies and, whenever appropriate, exploits the unique resources in the physical sciences found throughout the Los Alamos National Laboratory.

Life Sciences Division consists of four technical groups: LS-1, Physiology; LS-2, Biochemistry/Biophysics; LS-3, Genetics; LS-4, Cell Biology. These groups are responsible for 85 projects. Support for the division's projects is largely from the Department of Energy's Office of Health and Environment Research (OHER). A significant portion is derived from the Department of Defense (DoD) and the National Institutes of Health (NIH). Institutional Supporting Research and Development (ISRD) funds are also used to support new initiatives. Projects funded by all these sources are included in this report because they are integrated into the Division's strategic plan and serve to strengthen and complement the core OHER effort.

Energy and Health

The primary mission of the DOE is to ensure the energy needs of the nation. Major sources of energy are from nuclear fission and fossil fuel. Both processes generate byproducts that can affect health largely through the induction of carcinogenesis caused by DNA damage. As a consequence of these concerns a major objective of the Life Sciences Division is to understand the health effects of different radiations and chemicals. The basic biological principles will apply irrespective of whether the radiation and chemicals are manmade or from natural sources. Research projects in the Division address the molecular mechanisms of mutagenesis, DNA repair and the regulation of gene expression. A recent success of the Division has been the cloning of the human excision repair gene ERCC-5. This gene is being characterized. Because carcinogenesis is characterized by genetic instability and aberrant cell growth, research projects are further directed to understanding cell cycle controls, cell metabolism, differentiation and the processes of tumor growth and tumor rejection. These projects use the latest technologies of molecular and cellular biology; recombinant DNA technology, tissue culture, DNA sequencing oligonucleotide synthesis; cytogenetic analysis, cell synchronization, flow cytometry (FCM); pulsed-field electrophoresis and high performance liquid chromatography. The National Flow Cytometry Resource located in the division provides unique FCM instrumentation for biomedical research programs and chromosome sorting for the Human Genome Project. All efforts are made to keep the FCM instrumentation at the highest level of performance by incorporating advances made in this division and in Lawrence Livermore National Laboratory. New data acquisition systems, incorporating cutting edge electronics, computer and programming techniques, are being developed. Under investigation is a new approach to cell and possibly subcellular manipulation by using the radiation forces of laser beams to optically trap cells and organelles.

The Human Genome Project is part of a nationwide effort to physically map the 100,000 or so human genes and eventually to sequence human genomes. The project will provide the DNA sequence data base for biomedical research concerned with DNA damage from radiation and chemicals and with human susceptibilities to genetic diseases. Major research activities in the LS Division are directed to chromosome sorting, to developing human chromosome libraries, and to the physical mapping of chromosome 16 and other chromosomes. Cosmid and phage libraries of chromosome 16

have been constructed and characterized and concise ordering of chromosome 16 loci has been initiated using large DNA fragments from the specific libraries. In collaboration with CLS Division a major effort is under way to develop advanced DNA sequencing methods based on single molecular detection. The high-risk, high-return venture has the potential to revolutionize DNA sequencing methods.

Whereas radiation-induced DNA damage is largely a random hit mechanism this is not the case for chemically induced DNA damage that is determined in part by the accessibility of the DNA substrate to the chemicals. Thus understanding chemical damage to DNA will require an understanding of the packaging of DNA in chromosomes and changes in chromosome structure through the cell cycle. It is remarkable that although chromosomes have been central to biology since they were first observed almost two centuries ago surprisingly little is known at the molecular level of their organization, structure, and functions. Such understanding is central to basic questions in biology, e.g., developmental cell biology, as well as to the mission of the DOE. Major projects in the LS Division are concerned with the organizations of chromosomes, particularly the functions of repetitive DNA sequences. Following up the Division's success in the identification of the human telomere DNA sequences, studies are now directed to the characterization of proteins that bind specifically to these sequences and to the isolation of human telomerase that maintains the telomeres.

The DNA sequence data base from the Human Genome Project is important for two major problems in structural biology. The first is the DNA organization, structure and functions of chromosomes at all levels down to the nucleosome. The second is eventually to have the capabilities to go from linear DNA gene coding sequences to three dimensional protein structures and their functions. The latter will require an enlarged protein sequence-conformation data base. To approach these long-term problems the DOE has proposed a structural biology initiative that will utilize the unique facilities in the National Laboratories such as high flux neutron and X-ray beams and high speed computers. To take advantage of this initiative we have to build on the current success in structural biology in determining the solution structure of calmodulin by X-ray and neutron scatter. It will be necessary to develop in-house capabilities in X-rays and high resolution nuclear magnetic resonance spectroscopy that will complement the high flux neutron and X-ray sources. Because we lack even low resolution solutions to many of the structural questions concerning the folding path and packaging of DNA in chromosomes, imaging techniques with the potential to image hydrated states of nucleoproteins will have to be evaluated. Such techniques are scanning tunneling microscopy (STM) and atomic force microscopy (ATM), a derivation of STM. Although it has been proposed that STM has potential for DNA seqencing this has yet to be demonstrated. However, "pictures" of DNA have been published by Lawrence Livermore National Laboratory and Lawrence Berkeley Laboratory.

The radiation biology program in the Life Sciences Division focuses on determining the molecular mechanisms of radiation damage in living organisms and the limits and consequences of low levels of radiation exposure. Projects concerning the mutagenicity of low doses of ionizing radiation address the problems of identifying the primary genetic lesions of radiation damage. Projects concerning the effects of radiation on chromatin structure address issues involving the role of protein-DNA interactions in radiation damage. Cytogenetic and flow cytometry studies on chromosomes are used to develop new methods of assessing radiation damage. The mechanisms of radiation damage are also studied at the cellular level using cultured cell models. These programs on low-level radiation effects provide an important data base for the assessment of national concerns about nuclear reactor accidents such as Three Mile Island and Chernobyl and fulfill the need to understand the consequences of radiation exposure resulting from national defense activities.

Other programs in the Division focus on determining the molecular and cellular mechanisms by which chemicals and elements from conventional fuel sources may result

in health hazards. Genetic studies are conducted to understand the mutagenicity of fugitive chemicals generated by energy production. The mechanism of action of heavy metals, mobilized to the environment by energy production, is being investigated. The mechanisms of toxicity of these substances are being studied in cultured cell systems, with particular focus being placed on the metal ion disruption of normal DNA-protein interactions, the subsequent induction of genetic activity, and the resulting consequences on cell proliferation.

The exposure of whole animals to potential toxic substances of the energy industry is being conducted in the Division's inhalation toxicology laboratory to determine the mechanisms of lung damage and recovery. The effects of toxic substances on lung cell-cell interactions and lung cell-matrix structures are also being studied in model lung cell cultures to determine the biochemical and cellular targets of these toxicants. Such studies provide a base of knowledge for accurately assessing health issues that might arise in energy industries and provide guidance for designing safe energy production processes. These studies will also provide a base of knowledge for assessing the impact of energy production on the environment.

Other environmental concerns are also addressed by the Division's plant stress programs. The reclamation of land disturbed by energy production activities will require understanding of chronic effects of toxicity on vegetation and the mechanisms of salt stress and salt tolerance in plants. An example of basic researach having spin-off into the problems of environmental clean-up is provided by plant genetics and molecular biology studies of *Datura innoxia* cells (jimsonweed). This is a model system used to understand molecular mechanisms underlying environmental stress in higher plants. The peptides, poly(γ-glutamylcysteinyl) glycine from these cells have been shown to bind Cd^{2+}; the structure/function relationships of this binding process are an integral part of the program. Practical applications that derive from the basic study are the use of resin immobilized peptides to bind Cd^{2+} and other metal ions; the use of *Datura innoxia* cells to bind Ba^{2+} and Pu^{4+} and remove those ions from laboratory waste. These cells also have the ability to take up 2,4,6 trinitrotoluene (TNT) and other explosive compounds from solution.

The Life Sciences Division has active programs in physiological biophysics that draw on physics strengths in the Laboratory. Many diagnostic techniques in medicine use basic physical principles, e.g., X-ray scanning and ultrasound. In a collaboration with Physics Division, magnetoencephalography (MEG) is being applied to the "imaging" of metabolic activity in the brain. Such studies have applications to both normal brain function and to the diagnosis of brain disorders as manifested by metabolic malfunctions. A relatively recent addition to techniques of physical diagnosis is magnetic resonance imaging (MRI). MRI has particularly powerful application in the identification of brain lesions that are not observed through X-ray scanning. By the use of stable isotopes MRI and *in vivo* nuclear magnetic resonance (NMR) can be used to monitor disease related changes in concentrations of metabolites. The Life Sciences Division has active programs concerned with other diseases. Biochemical research is conducted on diabetes and gonadal hormones using flow cytometry and chromatography. Neurobiology projects are focused on mental illness, neurological disorders and neuroendocrinological problems. The application of physical techniques in physiology and medicine is a major interface for physics and life sciences.

National Security

Life Sciences Division provides the nation with a technological base for several national security programs in the life sciences. The pulmonary toxicology program studies the effects of NO_x exposure in animals to determine the mechanism of pulmonary injury by NO_x inhalation and to generate a data base to assess health effects on soldiers in the field. These programs use our latest advances in cardiopulmonary physiology and the National Flow Cytometry Resource.

Flow cytometry has also provided the basis for the development of a new technology for ultrasensitive analysis. This technology is relevant to the national security need to analyze low levels of toxins and chemical agents. Laser light scattering technology has been applied to a national security program to develop LIDAR, a technology for the rapid identification of bacteria in the air.

Another national security program focuses on performance enhancement. The projects in this program use automated behavior analysis, electrophysiology, neurochemistry, NMR, and MEG to study the neural processes underlying task performance and the adaptation to stress.

Our genetics program strengthens our national security with its plant genetics projects that use cell culture and recombinant DNA technology to genetically engineer plants for tolerance to arid regions. These projects increase our national security by providing the technological base for developing food production under adverse agricultural conditions and for maintaining American competitiveness in the agriculture sector.

In conclusion, Life Sciences Division uses its biomedical technology base to support the mission of both the Department of Energy and the Los Alamos National Laboratory, which is to conduct basic and applied research of importance to the nation's security, with emphasis on defense science, technology, and energy needs.

PHYSIOLOGY GROUP

LS-1, the Physiology Group, conducts basic and applied research directed at understanding the function of integrated biological systems. This level of study complements and also benefits from the efforts of the other groups whose investigations are carried out at the molecular, cellular, and biochemical levels. The Physiology Group is subdivided into two sections: Neurobiology and Pulmonary Biology. The work carried out in these sections supports the missions of the Laboratory and of the Life Sciences Division by addressing research questions that are relevant to human health, to the effects of energy production, and to national security. A brief description of the goals and interests of each section follows.

Neurobiology

The focus of neurobiological research in LS-1 is in the area of neural processing. The general goal is to understand the analysis and transformation of signals by integrated systems of neurons. The signals that are processed may be physical, such as light or acoustic waveforms, or they may be outputs of other neural systems, as in studies of selective attention or behavior. Some of the basic research projects currently under way include studies of the auditory processing of human speech, studies of auditory and visual attention using magnetoencephalography (MEG), biochemical and biophysical studies of phototransduction, and studies of the neuropharmacological and behavioral effects of drugs used to treat psychiatric disorders and drug abuse. In many cases the Laboratory's strengths in physics, computation, and theory have been brought to bear on studies of neural processing; the development of the MEG facility by Dr. Ed Flynn of the Physics Division is an excellent example.

Pulmonary Biology

The focus of pulmonary research is on identifying and understanding the mechanisms of lung damage and disease progression that may follow inhalation of environmental agents. Substances that result from energy production or from military activities have been targeted for study. This work involves collaboration with other investigators from the Life Sciences Division and makes extensive use of the National Flow Cytometry Resource in LS-4. In addition, the Pulmonary Biology Section maintains an Electron Microscopy Facility that can be made available to other

projects within the Division.

BIOCHEMISTRY/BIOPHYSICS GROUP

The Biochemistry/Biophysics Group (LS-2) comprises three scientific sections: Biochemistry, Biophysics, and Structural Biology.

Projects in the Biochemistry section have a common theme of protein biochemistry with an emphasis on interactions between proteins and DNA. A general interest of one project is the structure of chromatin and the role that histone modification plays in the condensation of chromatin. Studies of histone modification are facilitated by recently developed high performance liquid chromatography (HPLC) techniques for the analysis of nucleosomal core histones and non-core nucleoproteins and the application of these techniques to small numbers of cells. A second project is concerned with the proteins that have been found to bind to repetitive DNA sequences specific to the pericentric region of human chromosome 9. The current direction of this project includes the characterization of the proteins and investigation of the DNA-protein binding interactions. The development of technology for the analysis of protein mixtures is the focus of another project in the biochemistry section. At present, the studies on this project cover methodology for the separation of protein mixtures by two-dimensional gel electrophoresis and characterization of the protein mixture by either radioactivity counting or staining with fluorescent dyes. The disease of diabetes mellitus is investigated in two projects. Renal biochemistry studies investigate the kinetics of the non-enzymatic glucosylation of plasma albumin and the urinary excretion of glucosylated albumin as a measure of the functional state of the glomerulus and of general metabolic function. These measurements in diabetic individuals, who show enhanced urinary excretion of glucosylated proteins, can be used to study re-adsorption mechanisms. Red blood cell biochemistry studies focus on the recent observation that reduction of Na-K ATPase activity in the diabetic red blood cell results in swelling of the cell. This increase in the size of the red blood cell can be responsible for the circulatory disorders in diabetic individuals. Several projects are concerned with microbial biochemistry. One general area of interest in these projects is the environmental mobilization of actinide elements by microorganisms. The present work in this area is concerned with the isolation, characterization, and chemistry of microbial chelating agents. A second area of investigation is a preliminary study on the microbial degradation of organic materials. There are two projects that combine nuclear magnetic resonance (NMR) spectroscopy techniques and stable isotope technology in order to address problems in biochemistry. One project seeks to develop non-invasive NMR methods that can be applied to biomedical research. These studies currently include NMR characterization of prostate tumors and the development of techniques for NMR imaging of the carbon-13 nucleus with sensitivity comparable to that of the proton. The second project employs NMR spectroscopy to study the structure of plant cell walls.

The Biophysics section of the group is concerned with the development of optical instrumentation and the application of this instrumentation to biological problems. One such application has been the use of light scattering techniques to investigate and characterize bacterial and mammalian cells at a gross structural scale. Our most recent light scattering studies have been the measurement of phase differential scattering patterns and morphological characterization of subsurface microorganisms. There continues to be an active effort in the biophysics program to develop theoretical models for light scattering by irregular and chiral biological structures. Another application has been the development of a light detection and ranging (LIDAR) system for the remote detection of airborne bacterial and viral particles. The initial application of a newly developed Zeeman interferometer is also the detection of airborne microorganisms; however, the technique should also be applicable to other ultrasensitive detection methods in both air and water. The last

general area of interest in the biophysics program is the application of artificial intelligence technology to modeling problems in biology and to the analysis of multivariate flow cytometry data.

Studies in the Structural Biology section focus on the structure and dynamics of biological macromolecules and macromolecular assemblies in order to form a basis for understanding their function. The techniques of small-angle neutron scattering, small-angle X-ray scattering, Fourier transform infrared spectroscopy, and circular dichroism have been employed for structural studies. Inelastic and quasielastic neutron scattering experiments probe the dynamics of biological macromolecules. Neutron scattering experiments are conducted at the Los Alamos Neutron Scattering Center (LANSCE), which is the highest-intensity pulsed-neutron source in the United States. Previous studies in the structural biology program have included investigations of both the structure and the low frequency vibrational motions of cytochrome-c. Current studies are focused in three areas: investigations of antibody-antigen complexes to elucidate the structural features that define antibody subclasses; studies of the structural basis for regulation of calcium dependent biochemical processes; use of ferrofluids for aligning biological assemblies to facilitate structural studies.

GENETICS GROUP

The primary objective of the Genetics Group (LS-3) is to examine the structure and function of the human genome. Special emphasis is placed on understanding (1) the evolution of DNA as a coding macromolecule, (2) the mechanisms underlying differential regulation of gene expression, and (3) the ways alterations in genome organization or gene expression underlie human disease. Our approach utilizes a wide variety of cell biology, biochemical, biophysical, and recombinant DNA techniques. An understanding of the mechanism(s) by which higher organisms organize and regulate the "readout" of specific genes is vital to a molecular understanding of diverse biological processes such as the development of a human embryo or the function of the adult brain. Equally as important, alterations in the normal information flow from DNA are thought to underlie most pathological states, from environmental toxicity caused by energy-related pollutants to the devastating, lethal changes found in cancer cells. Our program, therefore, meets both the DOE's and the Laboratory's need to understand the biological effects of energy-related technologies. In addition, it uses the Laboratory's unique expertise in both biophysical and molecular biological techniques to provide innovative technology transfer to the growing biotechnology industry. These goals are addressed by investigations in three specific areas.

First, our studies of human genome organization center on the identification, isolation, and understanding of specific DNA sequences that are involved in mediating chromosome structure and/or regulating differential gene expression. The first goal of this program has been accomplished, with the identification and cloning of the human telomere. A major project to construct recombinant DNA libraries from individual human chromosomes is now well under way. This program, established in collaboration with the Cell Biology Group (LS-4), provides a national resource from which investigators at the national laboratories, as well as worldwide, may obtain DNA sequences useful for studies in genetic mapping, chromosome structure, disease diagnosis, and the development of sensitive assays for DNA alteration. An exciting new initiative to construct long-range maps of human DNA has begun with emphasis on determining the physical organization of a single human chromosome.

Second, heavy-metal induction of the synthesis of metallothioneins continues to provide an ideal model system for basic mechanistic studies of gene expression. A combination of recombinant DNA and pulse-field electrophoretic techniques is used to study (1) gene structure and replication, (2) gene amplification, and (3) the control of RNA transcription and processing. Ultimately, an understanding of the mechanism

responsible for the coordinate regulation of this metal-induced gene family will allow realistic assessment of the health effects of energy-related pollutants. In this area, a new plant biotechnology program has been initiated, centering on the study of (1) heavy-metal metabolism and the isolation of metal-regulated genes in plants, and (2) plant salt stress tolerance.

The third major area of research endeavor is directed towards the isolation and characterization of human genes involved in the repair of DNA damage, the characterization of DNA structural changes induced by physical and chemical carcinogens, and the elucidation of the molecular mechanisms by which these structural changes are converted to heritable genetic changes. In related studies, the cytotoxic and genetic effects of chronic exposures to low doses of ionizing radiation are being examined. We believe that this integrated program will provide novel insights into the molecular and cellular responses mediating the physiologic effects of environmental carcinogens.

In summary, the Genetics Group has a variety of well-integrated programs designed to address major problems in genome organization, gene expression, and DNA damage and repair. Our Group provides cell culture and molecular biological facilities to the Laboratory and has developed collaborative studies with other divisions throughout the Laboratory. We have assembled an innovative staff and technical support team to apply cell biology, molecular biology, and recombinant DNA techniques to vital multidisciplinary problems. This cooperative venture is feasible only in a national laboratory, where technology sharing is encouraged and a clear vision of future direction and accomplishments is possible.

CELL BIOLOGY GROUP

The mission of the Cell Biology Group (LS-4) is to provide a multilevel understanding of the growth and differentiation of cells under both physiological and abnormal conditions. Experiments are currently under way at cellular, biochemical, and molecular levels using a wide variety of technologies and model systems. The mission of the group also includes the development, refinement, and application of optically based biophysical techniques, largely involving flow cytometry.

The National Flow Cytometry Research Resource, which resides in LS-4, is a user-oriented facility that makes unique instrumentation and expert assistance available to the biomedical research community. Capabilities include (1) triple-laser, eight-parameter flow cytometer with four-way sorting; (2) chromosome slit-scanning; (3) high-speed sorting; (4) chromosome sorting; and (5) multivariate data analysis. The Resource provides these advanced flow cytometric capabilities to the biomedical community in the form of sabbaticals, short-term visitations, consultations, collaborations, and teaching programs. Our dissemination program includes the Flow Systems Newsletter, a flow cytometry course presented to scientists from around the world and a course for local high school students, organization of meetings and workshops, and numerous tours of facilities throughout the year. Facility collaborators bring in new research dimensions, applications, insights, and experience, thus ensuring our continued leadership role in this field.

Because of the inherent complexity of the biological problems being approached using flow techniques, there is an increased use of multiparameter analysis wherein several electro-optical parameters are combined with fluorescence measurements to resolve unique cell populations. These parameters include electronic cell volume, axial light loss, low-angle light scatter, orthogonal light scatter, and multiwavelength autofluorescence measurements. To accommodate all these measurements, we have implemented "pipe-line" processing electronics to correct for the dead time that occurs between the sequential points at which measurements are made.

The analysis of multiparameter flow-cytometric data is extremely complex and time consuming. Accordingly, we are developing cluster analysis software, which will

simultaneously resolve unique cell populations in multidimensional space.

The cellular and molecular mechanisms of carcinogenesis are being addressed by asking what roles karyotype instability and cellular immortalization play in the evolution of the transformed and tumorigenic phenotype. One significant result of the work is that immortalization and the onset of karyotype instability appear to be critical, limiting events in the neoplastic process. Closely related studies involve understanding hormonal regulation of cellular growth in normal cells and characterizing alterations that occur during stages of neoplastic progression. It has become clear that an understanding of the processes that are responsible for the evolution of neoplasia can only be accomplished by appreciating the mechanisms by which normal growth and development are regulated.

The group is also dedicated to the study of fundamental radiobiology at the cellular and subcellular levels. Emphasis is placed on understanding how physical patterns of energy deposition from different forms of ionizing radiation interact with critical cellular structures (e.g., chromatin) to ultimately cause quantitative changes in the induced biological effect. In addition, through studies involving time-dose relationships, we also seek to learn how initially produced radiogenic lesions are modified by the cell. To date, our efforts have concentrated on high energy (660 keV) photons, 5 MeV alpha particles, ultra-"soft" (280 eV) characteristic X-rays, and the effects that these radiations have on cell inactivation (clonogenicity) and cytogenetic endpoints (e.g., metaphase aberrations and lesions visible in prematurely condensed chromatin).

In collaboration with Lawrence Livermore National Laboratory, we have completed Phase I of the National Laboratory Gene Library Project. All 24 chromosome types have been sorted at over 95% purity and complete digest libraries have been prepared. The American Type Culture Collection has taken responsibility for the upkeep and dissemination of the libraries to researchers throughout the world. We now have a high-speed sorting capability so that the Phase II Library Project can begin. We will sort large quantities of human chromosome 16, which will then be used to construct large-insert, partial-digest libraries that will be useful for ordering and fine-scale mapping.

Our ultrasensitive analysis program with Chemistry Division is designed to detect single molecules and has now reached that detection limit with the development of improved optics, a reduced sampling volume, and a photon-counting detector. This has provided a sensitivity 100,000 times greater than the best previously reported systems. In addition, proof-of-principle experiments, which show that this methodology can be used in the detection of antigen-antibody reactions, are now complete. Unlike other systems, the fluoroimmunoassay in a single-phase assay can be applied to small molecules like drugs or to very large biologically important molecules. The assays have a sensitivity in the range of 10^{-14} M.

SELECTED RESEARCH HIGHLIGHTS

1988 Life Sciences Division

Cloning the Human Telomere

R. K. Moyzis, J. M. Buckingham, L. S. Cram, L. L. Deaven, M. D. Jones, J. Meyne, and R. L. Ratliff

No one knows what, exactly, is at the tips of human chromosomes, or where, for that matter, the chromosomes actually end. Since the early 1970s it has been clear that the ends of chromosomes - the telomeres - must be different from the middle, otherwise the chromosomes would be whittled away with each successive round of DNA replication. But the nature of the telomeres - indeed, their very identity - has proved difficult to pin down. We now feel the long-sought human telomere, the piece of DNA that caps both ends of all 23 chromosomes, has been found. It is a sequence of six nucleotides, repeated over and over again, without a mistake. And if recent findings in protozoa are any indication, the telomere will be found to twist itself into novel configurations and form guanine-guanine bonds that defy the rules of DNA-binding laid out by Watson and Crick.

The human genome contains a variety of DNA sequences present in multiple copies. These repetitive DNA sequences are thought to arise by many mechanisms, from direct sequence amplification by the unequal recombination of homologous DNA regions to the reverse flow of genetic information. While it is likely that some of these repetitive DNA sequences influence the structure and function of the human genome, little experimental evidence supports this idea at present.

We reasoned, however, that evolutionary conservation of a particular repetitive DNA sequence family might imply that the sequence is essential to cellular function. To isolate highly conserved repetitive DNA sequences, we constructed a recombinant human repetitive DNA library (pHuR library, for plasmid human repeat). This library was constructed from randomly sheared and reassociated DNA, a method that minimizes the potential loss of sequences, such as centromeric repetitive DNA arrays, that are devoid of a given restriction enzyme site. Likewise, the ends of linear DNA molecules will, by definition, be unclonable after restriction enzyme digestion, but they should be represented in this library if they consist of repetitive DNA arrays.

Six plasmid clones that produced strong high-stringency hybridization signals with hamster repetitive DNA were isolated for further analysis. Four of these recombinants contained small DNA inserts (39-48 nucleotides), each containing a variation of the same alternating (dG-dT)·(dA-dC) sequence. This sequence, with the capacity to form the alternative Z-DNA configuration, is known to be ubiquitously interspersed in eukaryotic genomes and to be highly conserved. The other two clones, designated pHuR 93 and pHuR 143, consisted of 40 and 43 copies, respectively, of highly conserved tandem arrays of the hexadeoxynucleotide sequence TTAGGG. This hexanucleotide sequence is identical to the hexanucleotide sequence known to be at the telomeres of trypanosome chromosomes.

Clone pHuR 93 DNA was used to determine the chromosomal distribution of the $(TTAGGG)_n$ sequence in the human genome. Quantitative slot blot analysis, using flow-sorted human chromosomes, indicates that similar amounts of this repetitive DNA sequence are present on each human chromosome, regardless of the absolute chromosome length. Estimates of the amount of $(TTAGGG)_n$ sequences present in the human genome, determined from both quantitative hybridization analysis and the frequency of this sequence in the pHuR library indicate that 3000-12,000 base pairs (bp) (500-2000 hexamers) are present on each human chromosome.

The genomic location of the $(TTAGGG)_n$ sequences was further characterized by fluorescent *in situ* hybridization. To better control the *in situ* hybridization conditions, heptamers of the hexamers (GGGTTA) and (TAACCC) were synthesized and end labeled with biotin-11-dCTP. A mixture of these two probes was hybridized to denatured human metaphase chromosomes *in situ*, and fluorescein-labeled avidin was used to detect the biotinylated DNA. While either strand alone gives observable

hybridization signals, the fluorescent intensity increases when the two are mixed, presumably due to out-of-register concatenation. Fluorescence signals were observed at the telomeres of all human chromosomes. As shown in Figure 1 about 80-90% of the telomeres are clearly labeled in most metaphases. Some telomeres have very faint hybridization, but the intensity of label appears to be a random variation. The fluorescent label is at the very end of prometaphase chromosomes, but, as the chromosomes condense, counterstained chromosomal DNA can be seen beyond the labeled site. Whether this is the result of technical manipulation of the chromosomes or a function of chromosomal condensation remains to be determined.

To determine if the $(TTAGGG)_n$ tandem repeats are directly at the ends of human chromosomes, high molecular weight DNA was digested with BAL-31 nuclease for increasing amounts of time. This enzyme progressively shortens DNA molecules from their ends. Hence, sequences that are at the original chromosome termini will be progressively shortened, while internal DNA sequences will be unaffected by moderate digestion. Genomic DNA sequences complementary to clone pHuR 93 are devoid of most restriction enzyme recognition sites and remain as high molecular weight fragments after Sau3AI or RsaI digestion and gel electrophoresis. Digestion with BAL-31 nuclease prior to restriction enzyme digestion, however, shows a progressive shortening and eventual loss of over 99% of the genomic DNA sequences complementary to clone pHuR 93. The observed kinetics of BAL-31 digestion (200 bp/min) is consistent with our estimate that 250 to 1000 hexamers are present at each human telomere. The telomeric $(TTAGGG)_n$ sequences completely disappear after the removal of approximately 4000 bp.

Sequences complementary to a synthetic $(GGGTTA)_7 \cdot (TAACCC)_7$ probe are also present at the telomeres of other primates, mammals, birds, and reptiles. The evolutionary conservation of the $(TTAGGG)_n$ sequence, its terminal chromosomal location in a variety of higher organisms (regardless of chromosome number or chromosome length), and its similarity to functional telomeres isolated from lower eukaryotes, suggest that this sequence is a functional human telomere.

The recent finding that telomeric DNA oligonucleotides form intramolecular structures containing guanine-guanine base pairs has suggested that telomere function may involve novel DNA-DNA or DNA-protein conformations and interactions. The $(TTAGGG)_n$ repeat can prime, *in vitro*, the addition of *Tetrahymena* specific $(TTGGGG)_n$ repeats when the telomere terminal transferase of *Tetrahymena* is used. This priming ability suggests that the construction of mammalian cell artificial chromosome vectors, in a manner analogous to yeast artificial chromosome vectors, can now be attempted. Indeed, the production of stable acentric chromosome fragments in cancer cells may involve the natural yet inappropriate addition of telomeres to "tag" a fragmented region of DNA. Further, the identification of these $(TTAGGG)_n$ sequences at the extreme termini of human chromosomes will allow accelerated physical mapping of the human genome by using pulsed-field gel electrophoresis and partial digestion strategies. Strategies to isolate sequences directly adjacent to all human telomeres can now be pursued, which should facilitate the completion of a genetic linkage map of *Homo sapiens*.

Figure Legend

Figure 1 *In situ* hybridization of a biotin-labeled repetitive DNA sequence to the telomeres of human chromosomes.

Quantification of DNA Synthesis in Tumor Cells Selectively Identified by Immunofluorescence

H. A. Crissman, N. Oishi, and J. A. Steinkamp

A flow cytometric (FCM) multicolor fluorescence detection system has been used to measure the DNA synthetic activity in human colon adenocarcinoma (WiDr) cells pulse-labeled with the thymidine base analog 5-bromodeoxyuridine (BrdU). WiDr tumor cells were identified from normal human leukocytes in a mixture using a fluorescein-conjugated cytokeratin antibody and the DNA synthetic activity (S-phase tumor cells) was quantified by differential fluorescence analysis of BrdU/Hoechst quenching using the DNA-specific stains: Hoechst 33342 and propidium iodide. This new technique demonstrates the potential for quantifying the DNA synthetic activity in tumor cells by selective identification of the tumor cell population using fluorescent antibodies. This permits a more detailed approach for cytokinetic studies on the tumor cell population, especially in solid tumors that contain host inflammatory/stromal cells as well as tumor cells.

Recently we demonstrated a sensitive flow cytometric method that incorporates the principle of BrdU/Hoechst fluorescence quenching (1) for enhanced detection of BrdU-labeled DNA in cells treated for 30 min or less (2). The method involves the use of two DNA-specific fluorochromes: Hoechst 33342 (HO) and mithramycin (MI), a G-C binding dye whose fluorescence in the presence of BrdU remains stoichiometric to DNA content, under the conditions employed. Using differential fluorescence analysis, we showed that cells in S phase exhibited significant BrdU/Hoechst quenching and thereby produced MI minus HO difference signals that were proportional to the amount of BrdU-label. Bivariate distributions obtained were very similar to those shown previously for anti-BrdU/PI procedure (3), but since heat or acid denaturation is not required, cell loss is minimized and DNA and/or chromatin, RNA, histones, and other proteins including antigens are preserved. In addition, differential fluorescence analysis following two-color staining using Hoechst with either of the mithramycin-related compounds, chromomycin A3 and olivomycin, is also feasible. The addition of the RNA-probe, pyronin Y, to the MI-HO staining procedure also allows the assessment of RNA content and the DNA synthetic capacity of cell subpopulations. Another significant advancement in the procedure has been the use of propidium iodide (PI) or ethidium bromide (EB) in combination with Hoechst 33342 to quantify BrdU uptake. PI and EB, which are red fluorescent, DNA-intercalating fluorochromes, can be excited with Hoechst using a single uv or dual wavelength (uv/488 nm) sources. Dual-laser excitation permits correlated analyses of fluorescein (FITC) labeled cellular components (total protein and monoclonal antibodies to antigens) in conjunction with DNA synthetic capacity as illustrated below.

The instrumentation used in these studies was a multilaser flow cytometer (MPFC) (4). Cells were analyzed on a cell-by-cell basis as they passed through argon and krypton laser beams. The emitted fluorescence was measured using a multicolor fluorescence (5) and the corresponding PI and HO fluorescence signals were amplified and input to a differential amplifier for processing as PI minus HO differences. Figure 1 shows bivariate contour diagrams of the various parameters that can be measured by FCM on a mixture of human leukocytes and WiDr colon tumor cells that were pulse-labeled with BrdU and treated with FITC conjugated cytokeratin antibody. The mixture was then stained with HO and PI and analyzed by FCM. The HO vs PI-DNA content in Figure 1A shows the leukocyte population (L) well-resolved from the heteroploid tumor cells. Figure 1A also shows that the peaks of the G0/G1 and G2 + M-phase tumor cells (labeled 1 and 2, respectively) lie along a line, approximately 45 degrees with respect to the x-axis, that intersects the leukocytes and the zero axis, while the S-phase cells deviate from this line due to the quenching of HO by BrdU. The PI-DNA fluorescence, which is not affected by BrdU, remains proportional

to DNA content across the cell cycle.

The application of differential fluorescence (PI minus HO signals) analysis gives rise to zero difference values for G0/G1 and G2 + M cells, but positive values for S phase cells. The magnitude of the difference is directly proportional to BrdU incorporated during the pulse-labeling period. The bivariate contour for the PI minus HO difference measurements vs the PI-DNA content in Figure 1B shows the detection of BrdU-labeled, S phase cells in the tumor cell population.

The selective binding of anti-cytokeratin-FITC to heteroploid WiDR tumor cells is shown in Figure 1C. Antibody binding by leukocytes (L) was very weak by comparison. The capability for detecting and quantifying the relative percentage of cytokeratin-labeled tumor cells actively synthesizing DNA is demonstrated in the PI minus HO difference vs anti-cytokeratin-FITC bivariate profile (Figure 1D). In contrast to leukocytes (L), all tumor cells are cytokeratin positive, as verified also from Fig. 1C. The fluorescence difference histogram (y-axis) in Fig. 1D indicates that non-BrdU labeled tumor cells lie in the region designated UL, while cells actively synthesizing DNA can be easily discriminated from this subpopulation. Previous FCM studies using simultaneous FITC-cytokeratin labeling and PI-DNA content analysis, have shown the importance and illustrated the advantage and the potential clinical relevance (6).

These studies demonstrate the potential for separating host inflammatory cells from tumor cells and for selectively analyzing the DNA synthetic activity of tumor cells. Such analyses may have significant clinical relevance, particularly in solid tumors, where normal, infiltrating host cells are contained as well as tumor cells. Our technique can specifically detect and evaluate the DNA synthetic activity of all tumor cells in the S-phase compartment.

References

1. Latt, S.A. Microfluorometric detection of DNA replication in human metaphase chromosomes, *Proc. Natl. Acad. Sci.* USA **70**, 3395-3398 (1973).

2. Crissman, H.A. and J.A. Steinkamp. A new method for rapid and sensitive detection of bromodeoxyuridine in DNA-replicating cells, *Exp. Cell Res.* **173**, 256-261 (1987).

3. Dolbeare, F., H. Gratzner, M. Pallavancini, and J.W. Gray. Flow cytometric measurements of total DNA content and incorporated bromodeoxyuridine, *Proc. Natl. Acad. Sci.* USA **80**, 5573-5577 (1983).

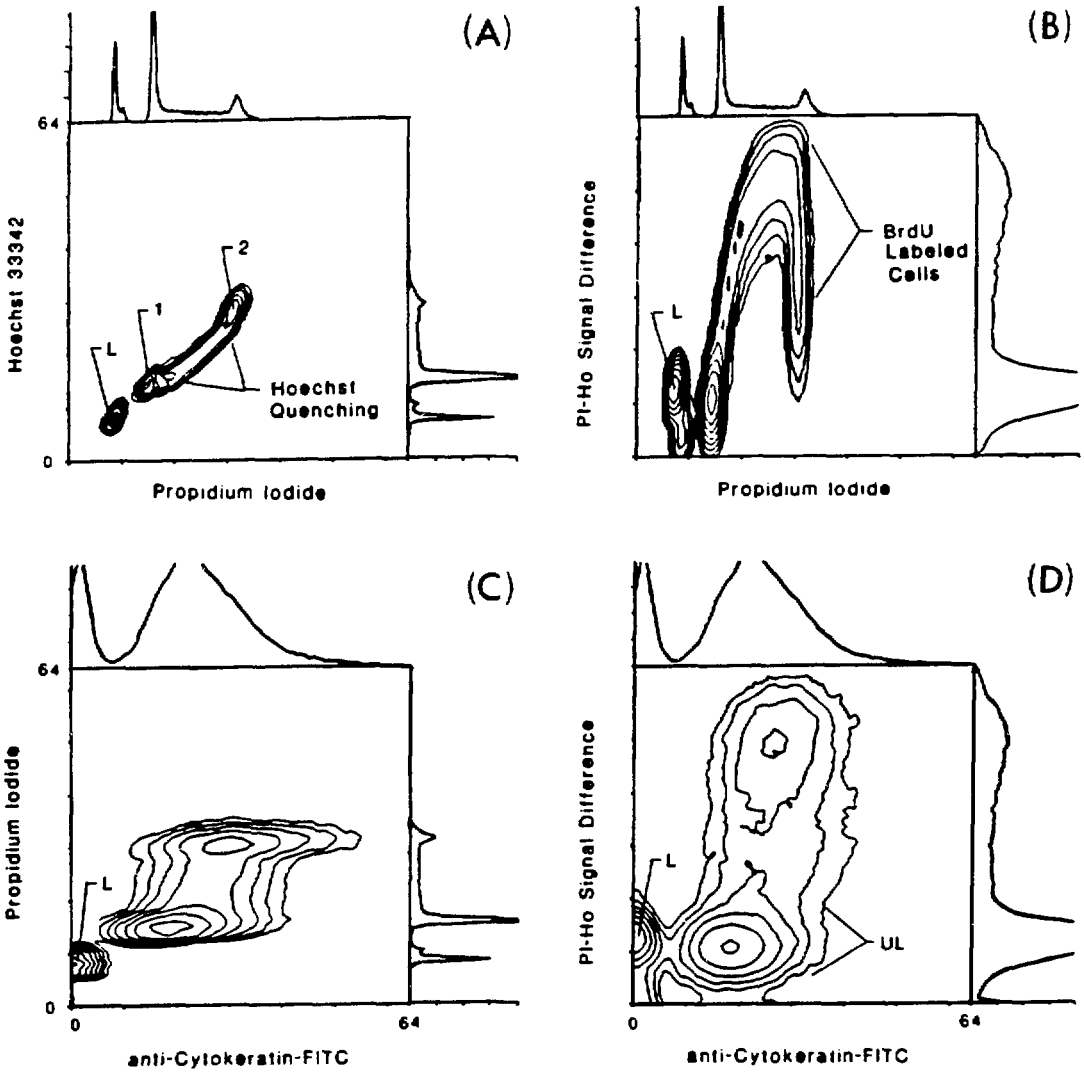
4. Steinkamp, J.A. Flow cytometry, *Rev. Sci. Instrum.* **55**(9), 1386-1389 (1984).

5. Steinkamp, J.A., R.C. Habbersett, and C.C. Stewart. A Modular detector for flow cytometric multicolor fluorescence measurements, *Cytometry* **8**, 353-365 (1987).

6. Oud, P.S., J.B.J. Henderik, H.L.M. Beck, J.A.M. Veldnizen, C.J. Herman, and F.C.S. Ramaekers. Flow cytometric analysis and sorting of human endometrial cells after immunochemical labeling of cytokeratin using a monoclonal antibody, *Cytometry* **6**, 159-164 (1985).

Figure Legend

Figure 1 Bivariate contour profiles for a cell sample (mixture) containing both BrdU pulse-labeled (30 μm BrdU, 1.5h) human colon adenocarcinoma (WiDr) cells and leukocytes from a healthy donor in a cell number proportion of 3 to 1, respectively. The sample was fixed in 70% ethanol labeled with anti-cytokeratin-FITC, and then stained with PI and HO. Shown are bivariate profiles for HO vs PI (A); PI minus HO signal differences vs PI (B); PI vs anti-cytokeratin-FITC (C); and PI minus HO signal differences vs anti-cytokeratin-FITC (D). Leukocyte subpopulations are designated L in all profiles; G0/G1 and G2 + M-phase tumor cells are labeled 1 and 2, respectively, in (A); non-BrdU labeled, cytokeratin positive tumor cells are designated UL in (D). All scales are linear. Contour levels were computer-generated for all profiles.



Data Acquisition Systems for Flow Cytometry

T. N. Buican, R. A. Habbersett, J. H. Jett, J. C. Martin, M. Naiver, J. Parson and M. E. Wilder

The development of a new data acquisition system for flow cytometers has been initiated. The new system will use state-of-the-art computer, electronics, and programming technologies. The new system will be the latest in a series of flow cytometric data acquisition systems developed at Los Alamos. The first specialized system for flow cytometry was put into use in 1974. It was capable of acquiring 8 bit data for 4 parameters. Sort decisions were made by computation. The successor to that system, the LACEL system, was placed in service in 1980. The LACEL system is capable of acquiring data on up to 8 parameters with 8 or 10 bit precision. Sort decisions are made in less than 10 microseconds by computer controlled, single channel analyzers and coincidence units. Copies of that system have been made by the Salk Institute in San Diego, the University of Helsinki, and the University of New Mexico. The software developed to control the data collection and for analysis of the data has been distributed to a large number of institutions and has formed the basis of a commercial product.

In addition, several specialized data acquisition systems have been implemented for one parameter data collection, one parameter sorting control, and for the acquisiton of fluorescence waveforms in the Fourier Transform Flow Cytometer (FTFC). The FTFC data acquisition system digitizes and records modulated waveforms. The system also deconvolves the waveforms in real time to provide emission spectral information or the emission intensities in selected spectral ranges.

The commercial computer bus standard, the VME standard, is being used as the backbone of the new data acquisition system. This standard provides room for the addition of user-defined bus lines. The user-defined lines will be used to communicate digitized data between the modules in the system. At the flow cytometer, the VME crate will have a local CPU, an ETHERNET interface, and the custom designed modules. In the initial configuration, these custom modules will include a digital pulse processor to calculate the integral, height and width of the fluorescence signals within 400 nsec of completion of the signal, a histogramming memory, and a bit-mapped sorting module. Data acquisition and sort decisions will be able to proceed at a synchronous rate of 300 kHz. The dedicated digital signal processing module will process the fluorescence signals at a rate of 60 Mbytes per second. This is a factor of more than 10 times faster than the LACEL system. By using a standard bus architecture, it will be possible to use commercial modules to drive displays and other functions. The VME crate will be connected to a powerful SUN work station via ETHERNET. The software to control the data acquisition system and provide a graphical operator interface will reside in the SUN workstation.

Structural Studies of Biological Assemblies Aligned Using Ferrofluids

J. Trewhella, T. Sosnick, E. M. Bradbury, R. Pynn, G. Stubbs, D. Gray, S. Charles, J. Hayter, P. Timmins

Scattering from isotropic solutions produces a one-dimensional data set containing limited structural information due to spherical averaging. Typically, solution scattering experiments can give information on the general shape or boundaries of the scattering particle only, and usually cannot prove a single model correct because more than one model can be consistent with the spherically averaged data. Scattering, or diffraction, from oriented particles in solution produces a two-dimensional data set that can provide more detailed information about molecular structure. In one exceptional case, that of tobacco mosaic virus (TMV), two- dimensional X-ray fiber diffraction data led to a complete three-dimensional structure of the virus at 3.6 Å resolution (1). The fibers consisted of bundles of the rod-shaped virus that were aligned using sheering forces. TMV has an intrinsic diamagnetic moment and can therefore also be aligned using moderate magnetic fields (1-2 T), as is the case for a number of biological structures (2). However, many biological structures do not have sufficient diamagnetic anisotropy to facilitate orientation in a magnetic field.

Extensive experiments have been carried out by Skjeltorp (3) to characterize the behavior of micron-sized, non-magnetic particles in a magnetized ferrofluid. Many of the results can be understood by noting that the non-magnetic particles introduce holes in the magnetized ferrofluid and that these behave as if they were magnetized in a direction opposite to the applied field in the absence of the ferrofluid. If the particles are rod-shaped with an axial ratio of at least 10:1, they will tend to align with their long axes parallel to the external magnetic field. The effective moment of the non-magnetic impurity is the product of the number of displaced ferrofluid particles and the magnetization of an individual ferrofluid particle. If large geometrically anisotropic biological assemblies could be aligned or forced into an ordered structure in a magnetized ferrofluid this could facilitate structural studies using scattering techniques, providing the ferrofluid particles could be "matched" to the solvent scattering density so that only the particle of interest was contributing to the scattering. Solvent matching can be achieved for neutron scattering by using deuterated components. Ferrofluid particles have been developed using a magnetite core and deuterated detergent surfactants that facilitate suspension in aqueous media. A non-ionic detergent surfactant is used to eliminate potential denaturation of the biological assemblies to be studied. Structural integrity of the assemblies in the presence of ferrofluid are initially assessed by electron microscopy.

Neutron scattering from TMV dispersed in deuterated ferrofluid (4) at 0.06 volume fraction and in the absence of magnetic field is isotropic (Figure a). A peak is observed corresponding to a regular spacing (363 Å) between the rod-shaped viruses due to electrostatic interactions. In a modest magnetic field (0.3 T) the scattering becomes anisotropic (Figure b), and the peak in the scattering appears only in the direction perpendicular to the field; although the magnetic strength of the field and the concentration of the virus are both well below the values where sheering forces or intrinsic diamagnetic moments would be sufficient to cause alignment (5). At higher scattering angles (Figure c), diffraction peaks can be measured that arise from the helical structure of the viral coat protein. A peak due to a characteristic repeat distance of 23 Å is observed to be oriented parallel to the direction of the field. This feature is expected from the known helical structure of the viral coat protein and confirms that the structural integrity of the virus has not been perturbed by the ferrofluid. Its orientation in the direction of the field is

expected for rods aligned with their long axes in the direction of the magnetic field.

Tobacco rattle virus (TRV) is genetically unrelated but morphologically similar to TMV. TRV shows only poor orientation under sheer, and does not align magnetically, even in a 7 T field at high concentration. However, when TRV is ferrodispersed at a volume fraction of 0.06, a field of 0.5 T is sufficent to produce alignment comparable to that obtained for the TMV, and a peak corresponding to a 25 Å distance arising from the helical structure of the coat protein is observed in the direction parallel to the applied magnetic field (Figure d) (5).

These experiments demonstrate that the ferrofluid system can be used to orient anisotropic particles independent of their intrinsic magnetic or sheer properties. Furthermore, the orientation is good enough to facilitate the measuremnt of internal structural parameters. In these examples, the structural integrity of the assemblies has not been perturbed by the presence of the ferrofluid. Future studies of the virus particles will focus on methods for improving the orientation so that higher resolution structural parameters can be determined.

Another application of the technique of future interest concerns DNA/protein assemblies. These assemblies are elongated structures that undergo dramatic rearrangements during function. Electron microscope studies of a number of DNA/protein assemblies in the presence of ferrofluid indicate they also maintain their native structures in the presence of the ferrofluid. Experiments are underway to study these systems oriented in order to determine the higher order structures and structural transitions that occur during replication and or transcription of the genetic code.

References

1. Namba, K. and Stubbs, G., *Science* **231**:1401, (1986).

2. Glucksman, M., Hay, R.D., and Makowski, L., *Science* **231**:1273, (1986).

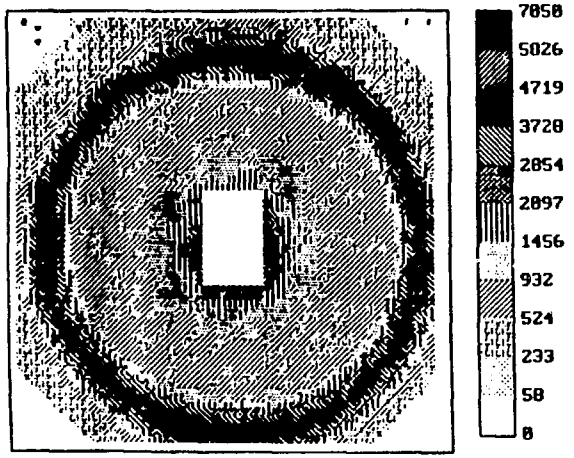
3. Skjeltorp, A.T., *Phys. Rev. Lett.* **51**:2306, (1983).

4. Oldenberg, R., Wen, R.B., Meyer, R.B., and Caspar, D.L.D., *Phys. Rev. Lett.* **61**:1851, (1988).

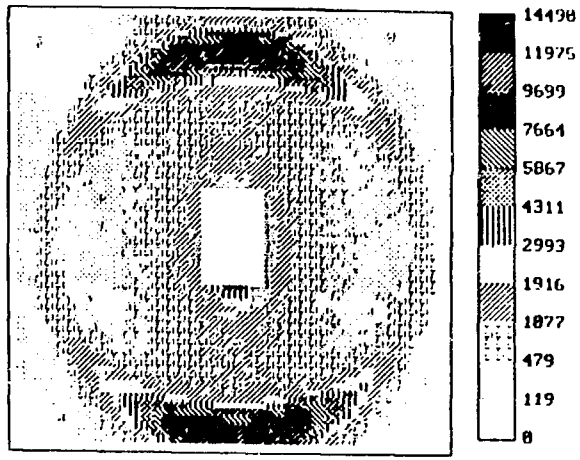
5. Hayter, J.B., Pynn, R., Charles, S., Skjeltorp, A.T., Trewhella, J., Stubbs, G., and Timmins, P., submitted to *Phys. Rev. Lett.*

Figure Legend

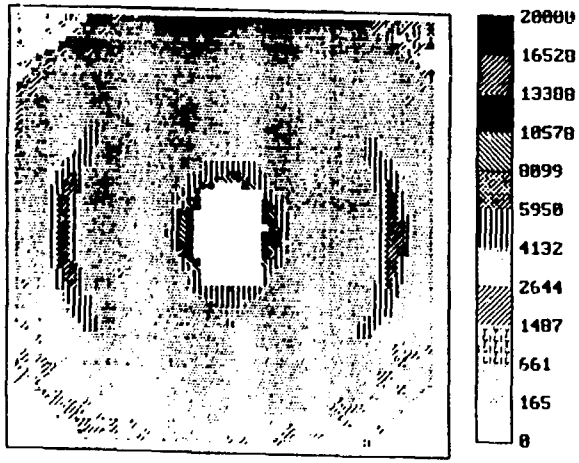
Figure 1 The upper two panels show small angle neturon scattering data from TMV particles dispersed in deuterated ferrofluid in the (a) absence and (b) presence of a magnetic field (0.3 T). The diffraction peak occurs at a scattering vector that corresponds to a repeat distance of 363 Å. The lower panels show higher angle neutron scattering data from (c) TMV and (d) TRV virus particles dispersed in deuterated ferrofluid in the presence of a magnetic field (0.5 T). The diffraction peaks occur at scattering vectors that correspond to distances of 23 Å and 25 Å, respectively.


7858
5026
4719
3728
2854
2897
1456
932
524
233
58
0

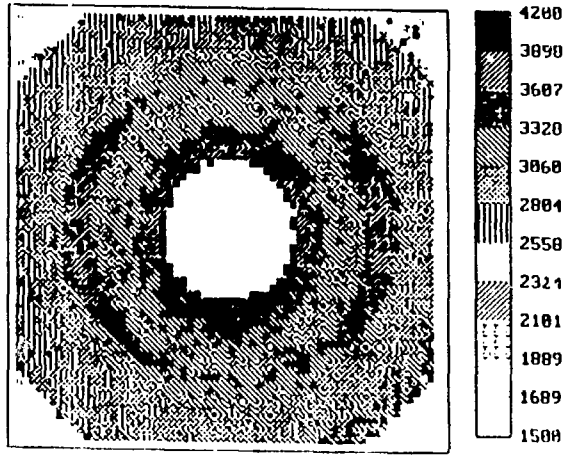
(a)


14490
11975
9699
7664
5867
4311
2993
1916
1077
479
119
0

(b)


20000
16520
13308
10578
8099
5958
4132
2644
1487
661
165
0

(c)


4200
3890
3607
3320
3060
2804
2550
2321
2101
1889
1689
1500

(d)

Human DNA Repair Genes: Cloning and Characterization

M. A. MacInnes, J. S. Mudgett, D. J. Chen, L. S. McCoy, and G. F. Strniste

Our biochemical knowledge of mammalian DNA repair mechanisms for processing of diverse DNA damages is very limited. Repair systems may have inherent constraints determined by the large size of mammalian genomes. DNA repair mechanisms may also have direct roles in the origin of certain genomic mutations, or in recombination. These fundamental unresolved questions require that we define biochemically the major repair protein components and their interactions with damage in the mammalian chromosomal milieu. The long term objective of these studies is to reconstruct physiologically the major repair enzyme-chromatin complexes, *in vitro*. With recent rapid advances of molecular cloning technologies, our immediate objective of repair protein characterization can be realized via cloning of specific human DNA repair genes in functional forms.

DOE-OHER sponsored efforts have been instrumental in the isolation of human ERCC genes (*Excision Repair Cross Complementing* Chinese hamster mutants) and XRCC genes that complement hamster X-ray repair deficient cells. At Los Alamos, we have employed this approach towards cloning the human *ERCC-5* and *XRCC-2* genes. ERCC-5 is essential for one or more steps in the nucleotide excision repair pathway. ERCC-5 has been previously mapped to human chromosome 13q by human-CHO cell hybridization studies. The human gene XRCC-2 encodes a function putatively involved in a repair pathway for double-strand breaks. Several studies have determined that it is part of a large genetic system, comparable to that of excision repair, involved in repair of X-ray DNA damage. However, a human chromosomal locus for the XRCC-2 gene has not yet been determined.

The human repair gene ERCC-5 was isolated at Los Alamos in December 1988. The gene was found by extensive functional screening of clones from a large cosmid DNA library derived from UV-resistant, human DNA-transformed CHO cells. Initial sorting of all cosmid clones containing human repetitive sequence by assignment of contiguous DNA overlaps (contigs) was crucial for our success. The repair gene is contained on one of the more rarely isolated contigs, and its activity is seen only when appropriate overlapping pairs of cosmids are cotransferred together into the CHO UV^s mutant. The cosmid pairs recombined during DNA tranfer to produce functional molecules which cotransferred UV resistance and a drug resistance marker (carried on the cosmid) at very high frequencies.

The cloned ERCC-5 gene was also identified as such by a second criterion: the inheritance of specific human DNA fragments. A human DNA probe from the active cosmids showed completely consistent inheritance with the UV resistance phenotype in all independent human DNA transformants tested, as well as in three human x CHO cell-cell hybrid cell lines used previously to assign ERCC-5 to chromosome 13. The functionality of the cosmids for transfer of UV resistance and the completely consistent inheritance of human DNA probes allow the firm conclusion that the ERCC-5 DNA repair gene has been cloned. Similar probes will be used to isolate the protein coding cDNA form of the gene for both sequence analysis and construction of expression vectors that will facilitate purification of the ERCC-5 protein.

What is the function of the ERCC-5 protein? CHO cells were the first recognized system to express a novel form of excision repair that is very selective for chromatin domains of actively transcribing genes. The panel of human ERCC genes/proteins probably participate directly in this damage recognition mechanism, which results in more efficient DNA damage excision in large genomes. We envision that ERCC-5 protein may show direct interactions either with damage sites, and/or DNA sequences localized in genes, or other proteins localized in active chromatin.

Our approach to isolate the XRCC-2 gene also employs human DNA transformation

into a CHO X-ray sensitive mutant. Human DNA and a marker plasmid DNA (pSV2gpt) are co-transferred to permit expression of the repair phenotype (bleomycin resistance) and the plasmid-bourne drug resistance (gpt^r) marker. We have analyzed human repetitive and pSV2gpt DNA sequences in a putative secondary XRCC-2 cotransformant DNA using Southern blots. The cotransformant contains roughly 50,000 base pairs of human DNA and 3 to 5 copies of pSV2gpt. The analysis of a large cosmid DNA library derived from the cotransformant is under way by the same screening methods as those applied successfully for the ERCC-5 gene.

Understanding the properties of the cloned ERCC-5 and putative XRCC-2 repair genes will be helped by the isolation of cDNAs that contain a copy of the entire coding sequences. The gene in this form is most amenable to DNA sequencing and deduction of its encoded protein sequence. A cDNA hybridization enrichment approach was designed to enhance the probability of cloning an active XRCC-2 cDNA. After several rounds of enrichment selection, based upon differential hybridization between cDNA from cells expressing XRCC-2 hybridized to mRNA from hamster cells not expressing it, the remaining cDNA was cloned into a mammalian gene expression vector. Reconstruction experiments to determine the actual enrichment factor of an unrelated cDNA (HGPRT) are in progress. The enriched cDNA library will be screened for functional clones by direct DNA transfer into CHO X-ray mutant cells. This protocol should allow isolation of any functional XRCC clone represented within the library.

The DNA repair pathways represented by the ERCC-5 and XRCC-2 genes constitute primary defense systems of human cells against many kinds of deleterious and potentially carcinogenic DNA damages. An understanding of repair protein interaction(s) with damaged DNA in chromatin should provide insights into fundamental mechanisms of carcinogenesis and cellular aging. Those repair factors which control repair fidelity and efficiency with respect to mutagenesis or recombination are of particular interest. This work will enhance our basic knowledge of the molecular and genetic factors determining human susceptibility to environmental radiations and genotoxic chemicals.

Bibliography

1. MacInnes, M. A., J. M. Bingham, L. H. Thompson, G. F., and G. F. Strniste. DNA-mediated cotransfer of excision repair capacity and drug resistance into Chinese hamster ovary mutant cell line UV-135, *Molecular and Cellular Biol.* **4**: 1152-1158 (1984).

2. Strniste, G. F., D.J.-C. Chen, D. de Bruin, J. A. Luke, L. S. McCoy, J. W. Mudgett, J. W. Nickols, R. T. Okinaka, J. G. Tesmer, and M. A. MacInnes. Restoration of Chinese hamster cell radiation resistance by the human repair gene ERCC-5 and progress in molecular cloning of this gene, in *Mechanisms and Consequences of DNA Damage Processing*, UCLA Symposia on Molecular and Cellular Biology, New Series, Vol. **83**, E. Friedberg and P. Hanawalt, Eds., (Alan R. Liss, Inc., New York), 301-306 (1988).

3. Mudgett, J. S., G. F. Strniste, and M. A.MacInnes. Cloning and partial characterization of the human excision repair gene ERCC-5, *J. Cell Biol.* **107**: 520a (1989).

4. McCoy, L. S., and D. J. Chen. Development of a novel procedure for cloning human DNA repair genes, *J. Cell Biol. 107*: 520a (1989).

5. MacInnes, M. A., and J. S. Mudgett. CpG islands and extent of CpG methylation in the promoter regions of three human DNA repair genes, in *Nucleic Acid Methylation, UCLA Symposia on Molecular and Cellular Biology*, in press (1989).

(^{13}C)-^{1}H Polarization Transfer Nuclear Magnetic Resonance Imaging of Metabolism

L. O. Sillerud, D. B. van Hulsteyn, and R. H. Griffey

We are pursuing the development and evaluation of advanced polarization transfer Nuclear Magnetic Resonance (NMR) techniques for imaging the distribution of ^{13}C-labeled substrates and metabolites in humans and other living systems. These images reflect the *function* of various organs, rather than their *structure* as revealed by the more traditional techniques of x-ray or ultrasound. Our methods will utilize two novel features of nuclear magnetic resonance. The first is the fact that we require no ionizing radiation, so that the procedure is virtually harmless. The second feature derives from our use of proton detection to increase the sensitivity of carbon (^{13}C) imaging above what would be possible through conventional techniques.

Polarization transfer methods elevate the sensitivity of ^{13}C NMR imaging to that characteristic of protons as a result of isotopic enrichment in ^{13}C and through indirect, proton detection of ^{13}C *via* ^{13}C-^{1}H J-coupling. They also enhance the specificity with which metabolic NMR images can be obtained by exploiting the large chemical shift range of ^{13}C in order to edit the proton signals to selectively image defined ^{13}C-labeled sites in biomolecules. Prior NMR imaging methods have primarily generated anatomical information derived from the bodily distributuion of water or lipids; our stated goal is the development of methods for generating functional images of molecular species of biomedical interest.

The basis of the technology is the detection of weakly magnetic ^{13}C nuclei through spin coupling to adjacent, covalently bound, more strongly magnetic protons. The sensitivity of direct detection of ^{13}C is only 1.6% that of the proton, and the natural abundance of ^{13}C is only 1.1%. Direct NMR imaging of ^{13}C at natural abundance is so time consuming (times of from 2 to 6 hours have been reported) that it is doubtful that it will ever achieve any medical relevance. The low natural abundance of ^{13}C can be circumvented through isotopic enrichment to levels greater than 99%. We are using indirect proton detection of ^{13}C to further raise the sensitivity of ^{13}C detection. Our preliminary ^{13}C-edited proton images have been produced in times on the order of 10 minutes with full proton sensitivity. This time is commensurate with the rates of metabolic processes in humans and other mammals, and raises the possibility that sequential indirect imaging of added ^{13}C would provide precise data with respect to the regional distribution of metabolic rates in organs of interest.

Source Localization of Components of the Visual-evoked Neuromagnetic Response

C. J. Aine, J. S. George, P. Medvick, M. T. Oakley, and E. R. Flynn

Measurement of the external neuromagnetic fields produced by electrical currents within the human brain is a powerful noninvasive approach for mapping spatial-temporal patterns of neuronal activation. Because this technique has good temporal and spatial resolution (of the order msec and mm), we have attempted to use the technique to discriminate patterns of activation in the human brain produced under different stimulus conditions. By using Magnetic Resonance Imaging (MRI), which provides detailed anatomical images of subjects' brains in conjunction with magnetic field measurements, evoked neural activity can be traced across cortical space and time.

Sinusoidal gratings of two or more spatial frequencies were presented randomly to different points in the visual field while subjects maintained fixation on a point in the central field. Neuromagnetic measurements were made in an aluminum and Mu-metal shielded chamber using an array of 7 Superconducting Quantum Interference Device (SQUID) - coupled gradiometer sensors. During a single trial-block, each stimulus type (e.g., a 1 cycle per degree grating at 2° in the right visual field) was presented randomly 25 times and the individual neural responses evoked by like stimuli were averaged together. The experiment was replicated 3 times for a single placement of the 7-sensor array. Field maps typically surveyed a minimum of 7 different areas on the head surface.

Amplitudes of the resulting averaged waveforms were measured from the mean prestimulus baseline and were sampled at 10 msec intervals. Field maps were prepared from the amplitude measures for each sampled time-point (see top row of Figure 1). If the field maps demonstrated two or more extrema of different polarity, a least squares procedure was applied to estimate the location and orientation of the source current dipole(s) and assess the "goodness of fit" between the empirical data and the hypothetical model. This algorithm solves the "inverse problem"—that is, it calculates the position and orientation of one or more current dipoles that best account for the surface data.

Upon examining the field distributions spanning 90-500 msec poststimulus, three distinct spatial-temporal patterns of activation were evident. A simple dipole-like distribution became apparent at 90 msec and was observed continuously through 140 msec (see lower left figure). However, the locations of field extrema systematically shifted during this time. Two other patterns were observed at 200-240 msec and 260-340 msec. The simplest explanation for these spatial-temporal patterns is that the location and orientation of a single current dipole source changed with time, producing a "path of activation" or "migrating source." However, similar field patterns might be produced by a linear combination of two nearby discrete sources whose relative strengths vary with time. For example, if one source was decreasing in strength while another nearby source was increasing in strength, the resultant extrema might appear as single peaks reflecting the centroids of positive and negative field distributions.

To discriminate between these possible explanations, computer modeling experiments were performed on the 90-140 msec sequence. In the first case, theoretical field distributions were calculated for each 10 msec time frame for a single source with location and orientation equal to the weighted average of these fitted parameters for the 90 and 140 msec distributions. In the second case, theoretical distributions were calculated by assuming a weighted linear combination of the 90 and 140 msec sources. The percent of variance accounted for by optimal versions of each of these models was compared. At every intermediate time-point, the single migrating source model produced a better fit for the data.

In conclusion, by using neuromagnetic measurement techniques in combination with MRI, we can noninvasively map visual function onto anatomical structure in humans. In many cases we have been able to identify one or more equivalent dipole sources that account for the evoked field distribution for a point in time following sensory stimulation. Computer modeling of experimental data can aid in discriminating between alternative physiological interpretations of the data. For example, in the case highlighted above, the data were best accounted for by a single migrating source model, which suggested that one group of neurons activated an adjacent group that then activated another in succession. The two discrete source models did not fit the data well. Therefore, it is likely that the observed activity was generated by a pair of sources activated by parallel thalamo-cortical projections with slightly different time-courses or by a discrete source occurring in one visual area that subsequently activates another visual area. The ability to make such discriminations will ultimately allow us to describe information processing activities of the human brain in considerable detail.

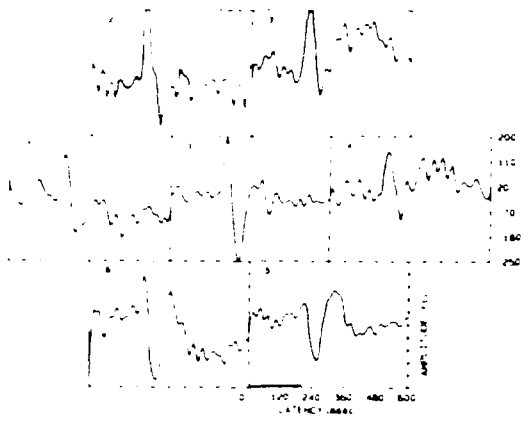
Figure Legend

Figure 1 Top left: The seven sensors are configured as the center and vertices of a hexagon as displayed here (2 cm separation). Each tracing represents an averaged evoked response for one stimulus condition. Top right: Amplitudes from each sensor location (49 sensor locations resulting from 7 placements of the 7-sensor array) were sampled at 10 msec intervals and field distributions were constructed for each point in time. Note the existence of a positive and negative peak in the field distribution constructed at 100 msec poststimulus. Lower left: Circles near the back of the brain (right side of image) indicate locations of calculated sources for the 90-140 msec sequence, when a 1 cycle per degree grating was presented 2° in the right field. For convenience the solutions were placed on one midsagittal MRI slice when in reality the sources were seen shifting back and forth between two different sagittal slices.

Figure

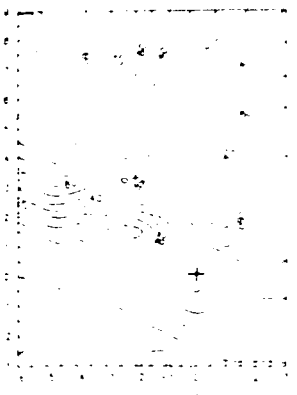
1. 7-SENSOR ARRAY

Sinusoidal Grating in Right Visual Field



2. CONTOUR PLOT

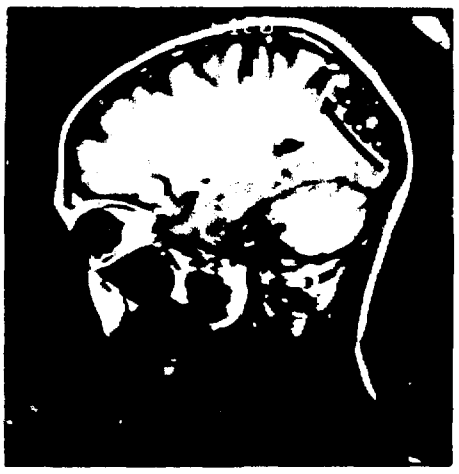
Magnetic Field Distribution -- 100 msec



Left Hemisphere Inion

3. MAGNETIC RESONANCE IMAGING (MRI)

Midsagital View



PROJECTS

ACCOMPLISHMENTS

GENOMIC STRUCTURE AND REGULATION

Project Descriptions:

Chromatin Structure During Replication (LANL)

Principal Investigator: J. A. D'Anna
Co-Investigator: R. A. Tobey, J. M. Gale

The objectives are to elucidate (1) the structural changes that occur in the mammalian genome during transient inhibition of DNA synthesis, (2) their underlying mechanisms, and (3) the mechanisms by which the structural changes might lead to cellular dysfunction. They are the result of observations at Los Alamos that (1) inhibition of DNA synthesis in early S-phase cells can cause enormous changes in the organization of chromatin, (2) the changes may be localized to initiated replicons, and (3) the change in organization can precede DNA synthesis in the normally early replicating replicons. Potentially, these organizational changes are important factors in the induction of genomic rearrangements, gene amplification and tumorigenesis. In addition, they should provide insight to replicon disassembly that is preparatory for DNA synthesis.

Protein-DNA Interactions (LANL)

Principal Investigator: R. L. Ratliff
Co-Investigators: D. L. Grady, R. K. Moyzis

The objective of this program is to investigate the mechanisms by which nuclear proteins recognize specific human DNA sequences. The binding of nuclear proteins to human chromosome-specific repetitive DNA sequences is investigated through the application of a gel electrophoresis DNA binding assay. Currently, this analysis is utilizing chromosome 9 specific human DNA repetitive sequences consisting of only alternating 10-mer and 5-mer repetitive domains, and the recently cloned human telomere repetitive sequence $(TTAGGG)_n$. Exonuclease III and DNase I footprinting have been applied to determine the exact binding region of the proteins to the repetitive DNA sequences. The human telomere sequence has been shown to bind a different protein from HeLa nuclear extract than the chromosome 9 sequence. We are currently purifying the telomerase from HeLa nuclear extracts using both standard biochemical techniques as well as DNA affinity chromatography. The telomerase adds repetitive (TTAGGG) sequences to the oligonucleotide $(TTAGGG)_7$ and could be useful in the construction of human artificial chromosomes.

Resource for Mapping of DNA Probes (NIH, DOE)

Principal Investigator: L. L. Deaven
Co-Investigator: M. L. Luedemann

This project provides a resource for mapping unique sequence DNA probes to specific human chromosomes. Labeled probes of unknown chromosomal origin are hybridized to spots of individual human chromosomes that were sorted onto nitrocellulose filters. The resulting autoradiographs reveal the chromosomal location of the probe. We are also conducting experiments on the use of slot blots for this approach to gene mapping. Slot blots could be made from pooled samples of

sorted chromosomes, thus reducing the sorting time required by filter handling and orientation. The resource provides a means of rapid gene mapping and has proved to be a valuable addition to our array of technical approaches to mapping the human genome.

A Physical Map of the Human Genome (LANL)

Principal Investigator: L. L. Deaven
Co-Investigators: C. E. Hildebrand, L. S. Cram

This project is designed to improve or develop technologies that are critical to the success of human genome research at Los Alamos. Currently, two areas of research have been identified that require developmental work to meet the needs of the genome project. These are the design and construction of an advanced programmable linear power amplifier for the analysis of parameters affecting large DNA fragment separation and the development of new chromosome-specific repetitive probes to improve the purity of partial digest libraries. An increased level of understanding of pulsed field gel electrophoresis should permit resolution of large fragments of DNA on the basis of their sequences as well as their sizes resulting in improved fragment resolution. Increased chromosome library purity will benefit work in a large number of laboratories in a variety of human genome research applications.

Chromosome Structure and Function (DOE)

Principal Investigator: L. L. Deaven
Co-Investigator: E. W. Campbell

The current aims of this project are to utilize fluorescent *in situ* hybridization techniques to improve the sorting purity of flow sorted chromosomes. Sort purity is a critical factor in the construction of chromosome enriched DNA libraries. We have adapted these hybridization methods to determine purity levels in chromosomes sorted from hamster-human hybrids or from human fibroblast or lymphoblastoid cells. The purity of DNA libraries produced here has a major impact on human genome research in a large number of laboratories; therefore, our efforts in this project to maintain state of the art sorter outputs have wide ranging beneficial impacts.

Human Recombinant DNA Library (DOE)

Principal Investigator: L. L. Deaven
Co-Investigators: C. E. Hildebrand, L. S. Cram

The goals of the National Laboratory Gene Library Project are the construction of recombinant DNA libraries for each of the human chromosomal types and the distribution of these libraries to user groups throughout the world. We have constructed a set of complete digest libraries for each of the human chromosomes and are currently working on a set of partial digest libraries. Libraries are distributed by the American Type Culture Collection, Rockville, MD. The libraries currently available have played a useful role in the search for markers for genetic disease diagnosis, and we believe the partial digest libraries will be invaluable tools for physical as well as genetic map constructions.

Study of Plant Stress (NaCl) Tolerance (DOE/OHER)

Principal Investigator: J. W. Heyser

An elucidation of the molecular mechanisms that confer increased salt (NaCl)-tolerance in halophilic cell suspensions and plants is sought. Ultimately an understanding of these mechanisms will aid in the transference of NaCl-tolerance from halophilic to nonhalophilic species. ^{23}Na, ^{13}C, and ^{15}N NMR spectroscopy is used to follow ^{23}Na transport and metabolism of ^{13}C and ^{15}N-labeled precursors to proline. Halophilic cells of *Distichlis spicata* and *Spartina pectinata* accumulate proline in response to salt stress as contrasted with NaCl-stressed cells of the nonhalophytes *Petunia hybrida* and *Panicum miliaceum*. The growth of *P. hybrida* cultures in high NaCl is stimulated by exogenous proline which suggests that differences in proline accumulation may partially explain differences in NaCl-tolerance. The isolation of pyrroline-5-carboxylate reductase, a key enzyme of proline biosynthesis, will soon be started so that the regulation of proline biosynthesis may be followed at a biochemical level.

Isolation and Characterization of Putative "Salt Tolerance" Genes from D. Spicata (USDA)

Principal Investigator: J. W. Heyser
Co-Investigators: H. J. Bohnert[1], Z. Zhao[1], R. Y. Johnson[2]

[1] University of Arizona
[2] Howard University

The isolation and characterization of genes that code for salt-tolerance are the major goals. cDNA libraries have been screened to isolate mRNAs which increase or decrease in response to NaCl. *In vitro* translation products of mRNAs from stressed and unstressed cells have been separated on 2D-gels to visualize changes in gene expression. Both procedures have shown that a small number of genes are either turned on or off by NaCl as evidenced by changes in relative transcript levels while the majority of mRNAs remain the same. Future research will be oriented toward (1) the isolation of promoters regulated by NaCl, and (2) the isolation of cDNAs which correspond to mRNAs which code for enzymes of the proline biosynthetic pathway.

Regulation of Gene Expression (DOE/OHER)

Principal Investigator: C. E. Hildebrand
Co-Investigator: D. L. Grady

The objectives of this program are to (1) define the structural and functional networks regulating eucaryotic gene expression at the molecular level, and (2) describe the interactions between DNA sequences and proteins in modulating gene structure and expression. Somatic cell, biochemical, and molecular genetic approaches are combined to study the expression of members of a multigene family encoding metal binding proteins (metallothioneins or MTs) involved in metal homeostasis and detoxification. These approaches have revealed several novel features of the organization and expression of the MT gene cluster as well as the dynamics of the intranuclear organization and replication of the MT gene cluster.

A variety of biochemical, biophysical, and recombinant DNA techniques are used to decipher specific DNA-protein interactions in modulating gene expression and chromosome dynamics. Our long-range goal is to determine if tandem arrays of

repetitive DNA located at or near centromeres or telomeres bind unique nuclear proteins in a sequence-specific manner, and to investigate the exact nature of this recognition and binding. These nuclear proteins could function in chromosome segregation during cell division through recognition and pairing of homologous chromosomes at the spindle apparatus during meiosis or mitosis. Alternatively, these centromeric and telomeric tandem arrays may bind classes of proteins involved in the higher order structure of DNA packaging, or in specifying the nuclear localization of specific DNA sequences.

Human Genome (DOE/OHER)

Principal Investigator: C. E. Hildebrand
Co-Investigators: R. L. Stallings, R. K. Moyzis, J. L. Longmire, L. L. Deaven

The goals of this project are (1) to develop concepts and to advance technology for genomic physical mapping, and (2) to construct a physical map of human chromosome 16 that will include an ordered set of overlapping DNA fragments encompassing the chromosome. The physical map will integrate restriction enzyme site locations, the genetic linkage map, identified gene sequences and the cytogenetic map into a tool that will allow rapid access to any region of chromosome 16 for analysis and eventual large-scale sequencing. Parallel and complementary approaches to constructing a low resolution physical map will employ a variety of resources and technologies: somatic cell hybrids for mapping subregions of chromosome 16, macrorestriction mapping, and chromosome-specific yeast artificial chromosome, cosmid and phage libraries as sources of probes for high resolution linkage maps and for developing overlapping clone maps of extended regions of chromosome 16. Optimal strategies for combining these approaches are based on computer analyses developed under a related project using novel algorithms both to generate simulated genomes (or chromosomal regions) and to execute various mapping strategies computationally. Collaborations with other DOE contractors and with the international scientific community have been initiated to: (1) accelerate mapping progress, (2) develop informational resources to compile physical maps and to interrelate them with linkage and cytological maps and sequence data, (3) improve the acquisition and analysis of physical mapping data through laboratory automation and advanced physical mapping technology. The significance of this work lies in the immediate application of the knowledge and tools (1) to understand human genetic disease, (2) to clarify the molecular bases for genetic disease susceptibility especially in regard to energy-related chemical or radiation exposures, (3) to reveal the molecular details underlying long-range chromosome 16 architecture and dynamics, and (4) to expedite the physical mapping of multiple human genomes and the genomes of other complex vertebrate species.

Plant Genetic Resources (LANL)

Principal Investigator: P. J. Jackson
Co-Investigator: E. Delhaize

Z. mays and tomato, two agronomically important plant species, have been genetically well characterized. The object of this project is to develop a molecular genomic map for these two species and relate this to the complex genetic map already available. Large numbers of metaphase chromosomes have been obtained from tomato cell cultures and from corn plant tissue. Efforts are now directed towards the purification of these chromosomes from other cellular components, quantitative staining of the DNA within these, and the flow-sorting and isolation of populations of specific plant chromosomes. Repetitive DNA probes have been isolated and will be used to study the distribution of this DNA among the different chromosomes.

Plant Genetic Resources (LANL)

Principal Investigator: P. J. Jackson
Co-Investigator: E. Delhaize

The goal of the project is to develop an understanding of the relationship among the physiological, biochemical, and molecular mechanisms of trace metal tolerance in higher plants. In an effort to determine the mechanism of metal tolerance, a comparison between tolerant and sensitive cells has been made. The amounts of metal-binding polypeptides, $(\gamma EC)_n G$, and the amounts of polypeptide/metal complexes, and of the immediate precursors to polypeptide synthesis were determined for Cd-sensitive and Cd-tolerant cells after the cell were exposed to normally toxic concentrations of Cd. Results demonstrate that the ability to produce large amounts of metal-binding polypeptides does not, in itself, confer tolerance to Cd, although the inability to produce these compounds results in rapid death of normally tolerant cells.

Plant Stress (DOE)

Principal Investigator: P. J. Jackson
Co-Investigators: E. Delhaize, N. J. Robinson

The objective of the project is to understand the genetic and biochemical mechanisms underlying tolerance to normally toxic concentrations of certain metal ions. Two-dimensional gel electrophoresis of *in vitro* translation products of mRNA isolated from Cd-tolerant cells exposed to Cd has allowed the identification of several different mRNA sequences. Cd has a similar effect on gene expression in Cd-tolerant and Cd-sensitive cells. However, there are two abundant mRNAs that are present constitutively in tolerant but not sensitive cells. These mRNAs encode proteins of ~11 kd and are either absent or present at low levels in sensitive cells. A comparison to *in vivo* labeled proteins suggests that either the mRNA s are not translated *in vivo* or they are rapidly turned over. Experiments to assay the enzymes involved in the synthesis of metal-binding polypeptides and their precursors are being used to understand the regulation of synthesis of these compounds.

Plant Strategies and Genetic Resources for Trace Metal Resistance (USDA)

Principal Investigator: P. J. Jackson
Co-Investigators: E. Delhaize, N. J. Robinson[1], P. Lammers[2]

[1] Durham University
[2] New Mexico State University

The goal of this project is to characterize the genes and gene promoters responsible for synthesis of mRNA sequences and proteins which are specific to Cd-resistant cells growing in the presence of Cd. This information will be used to develop an understanding of environmentally modulated gene expression in higher plants. cDNA clones have been isolated and are being characterized structurally. These are being used to identify genomic DNA fragments carrying promoter sequences. Promoters are being introduced into a "reporter" system, transformed back into plant cells and functionally characterized. Enzymes responsible for biosynthetic pathways known to be activated by exposure of cells to Cd are being identified and characterized, and the genes for these isolated.

Removal of Ba^{2+} from Complex Solutions Using Plant Cell Suspension Cultures (LANL)

Principal Investigator: P. J. Jackson

Ba^{2+} is found in waste streams generated during weapons production and explosives handling. It is also generated during the production of high temperature superconductor materials. This ion is toxic to humans. It is therefore required to be removed from effluent prior to environmental discharge. A series of chemical reactions is used to precipitate this ion from solution. The process is quite costly. An inexpensive, reliable method is therefore needed to replace current technology. Plant cells from suspension culture remove large amounts of Ba^{2+} from solution. Growth of cells in high concentrations of this ion results in the removal of Ba^{2+} to concentrations well below acceptable discharge concentrations. Addition of cells directly to waste solution also results in removal of this ion as well as several other complex molecules which are classified as environmental hazards. Removal of Ba^{2+} first occurs within five minutes after exposure to the cells. This ion forms a stable complex with a component of the cell wall. Barium comprises greater than 5% of the dry weight of the barium/cell complex. Methods to use these cells to remove Ba^{2+} and other complex molecules are being investigated.

Whooping Crane Program (DOI)

Principal Investigator: J. L. Longmire

The Patuxent Wildlife Center, in Laurel, Maryland, is currently attempting to captively breed the highly endangered whooping crane *Grus americanus*. Offspring produced in this project will be released to augment the diminished North American population. The purpose of our study is to genetically fingerprint each of the approximately forty cranes that constitute the Patuxent breeding stock. DNA fingerprints will be analyzed to allow researchers at Patuxent to pair cranes in a manner that will maximize genetic "outbreeding". By maximizing outbreeding, genetic diversity (and hence fitness) will be optimized in F1 individuals.

Falcon Genetics Program (DoD)

Principal Investigator: J. L. Longmire

The purpose of this program is to study population structure and migration in the endangered Peregrine Falcon *Falco peregrinus*. One goal is to develop a series of restriction fragment length polymorphisms to serve as population-specific markers. These markers allow investigations into gene flow occurring within and between geographically distinct populations. In addition, these markers will enable natal-origin determinations to be made upon individual falcons sampled during seasonal mass migrations.

Polynucleotide and Polynucleotide-Protein Structures (NIH)

Principal Investigator: D. M. Gray
Co-Investigator: R. L. Ratliff

The objective of this program is to determine if short interval stretches of protonated cytosine-cytosine ($C \cdot C^+$) base pairs could coexist with adenine-thymine ($A \cdot T$) base pairs. Future studies will be directed toward determining whether

oligonucleotide duplexes with $C \cdot C^+$ base pairs are parallel or anti-parallel stranded DNA.

Human Diploid Fibroblast Synchronization and Proliferation (DOE, LANL)

Principal Investigator: R. A. Tobey

The objectives of this program are to develop novel protocols for synchronizing the growth of cultured human diploid fibroblasts at multiple stages of the division cycle and to employ these synchronization protocols to elucidate mechanisms responsible for regulating genome replication. Studies with recently developed protocols focus on (1) molecular mechanisms controlling replication of early- and late-replicating genes; (2) chemical and physical properties of DNA replication complexes; and (3) replicon-wide alterations in chromatin structure during gene replication.

Genome Organization and Function (DOE/OHER)

Principal Investigator: R. K. Moyzis

The ultimate objective of this program is to determine the molecular mechanisms by which higher organisms organize and express their genetic information. Applications of these basic investigations will include the development of novel approaches for (a) the detection of human genetic diseases, and (b) measuring the effects of low-level ionizing radiation and/or carcinogen exposure. A combination of biochemical, biophysical and recombinant DNA techniques is being used to identify, isolate, and determine the roles of DNA sequences involved in long-range genomic order. Currently, major efforts are focused on (1) determining the organization and function of human repetitive DNA sequences, and (2) determining the factors regulating the organization and expression of metallothionein (MT) and related genes. Future studies will be directed toward (a) spatial organization in the MT multigene cluster, and (b) the definition and isolation of other "functional" repetitive DNA regions. Defining the mechanisms responsible for organizing the mammalian genome, and the genetic and nonmutational alterations accompanying abnormal phenotypic change are important aspects of defining the effects of energy related technologies. Determining the genetic variability in these mechanisms provides a rational basis for establishing thresholds for toxic substances, for making valid cross species extrapolations and, ultimately, identifying individuals at risk.

Interphase Analysis of Aneuploidy via in situ Hybridization (LANL)

Principal Investigator: J. Meyne
Co-Investigator: R. K. Moyzis

A major problem in the detection and measurement of genetic alterations induced by environmental agents has been the lack of sensitivity of the assays used. We are evaluating a new system for aneuploidy detection that may be more reliable and more sensitive than present methods. The ability to "tag" and quantitate specific human chromosomes, not only at metaphase but also in interphase nuclei, has been developed. A synthetic oligomer that hybridizes specifically to the centromeric region of chromosome 18 has been added to the battery of probes available, and a synthetic probe for chromosome 1 is being tested.

Research Accomplishments:

· Synchrony of Chinese hamster (line CHO) cells in early S phase was found to produce changes in nucleosome repeat lengths that precede DNA replication in the early replicating metallothionein II gene region; however, the changes do not remain fixed when cells are released to resume cell cycle progression. The potential reversibility of the system should be useful to understand normal replicon disassembly that occurs during unperturbed replication.

· A 13 kb region that includes the inactive CHO rhodopsin gene has been subcloned and mapped. It will be used to further test models of the changes in genomic organization that occur during inhibition of DNA synthesis.

· New methods for isolating DNA from flow sorted chromosomes with high efficiencies (90-95%) and with high molecular weight ($\geq$ 700 kb) have been developed.

· Ten genes of unknown chromosomal origin have been mapped to spots of individual human chromosomes sorted onto nitrocellulose discs.

· Relationships between humans and nonhuman primates have been determined using *in situ* fluorescence hybridization of total human DNA to chromosome spreads of five species of non-human primates.

· We have demonstrated that halophilic cells of *Distichlis spicata* and *Spartina pectinata* have NaCl-stimulated proline biosynthesis as contrasted with nonhalophilic *Petunia hybrida* and *Panicum miliaceum* cells in which NaCl inhibits proline biosynthesis.

· Three cDNAs which correspond to mRNAs induced by NaCl in *Distichlis spicata* have been isolated and are being sequenced.

· Using 2D-PAGE we have identified four proteins in *in vitro* translations which increase in response to NaCl, five which decrease, and nine which have transient increases during the initial 24 h of salt stress.

· Isolation and sequencing of the Chinese hamster MT I gene were conducted, and a novel computer analysis used to define regions of high homology with the human gene.

· Exonuclease III footprint analysis, band retardation assays, and *in vitro* synthesis reactions allowed us to synthesize proteins that bind to human centromeric and telomeric DNA.

· Macrorestriction analysis has been applied to reveal the organization of multiple classes of tandemly repeated DNA sequences encompassing the centromeric region of human chromosome 16.

· New methods have been developed for macrorestriction analyses of flow-sorted human chromosomes.

· Cosmid and phage libraries specific for human chromosome 16 have been constructed and characterized.

· Laboratory robotics instrumentation has been implemented to expedite preparative stages for generating overlapping cosmid contigs.

· Overlapping cosmid contigs spanning loci within band q22 of human chromosome 16 have been isolated.

· The mRNA sequences expressed in Cd-tolerant plant cells and in both tolerant and sensitive cells exposed to Cd have been identified. *In vitro* translations of these mRNA sequences demonstrate a range of mRNAs which are specific to cells exposed to Cd and several sequences which are specific to only tolerant cells.

· We have determined that the regulation of the synthesis of $(\gamma EC)_n G$'s appears to occur at the level of product biosynthesis, since the enzyme(s) responsible for synthesis are present in cells even in the absence of these metal-binding polypeptides. Glutathione was demonstrated to be the immediate precursor of metal-binding polypeptide synthesis.

· We determined that plant cell cultures can rapidly remove Ba^{2+} and explosive compounds from waste water produced during explosives handling and production. Barium binds tightly to a component of the cell walls.

· DNA fingerprints for forty-three cranes have been generated using the M13-related minisatellite probe. Computer algorithms have been developed to allow assessments of relatedness to be made from raw fingerprint data.

· Using DNA probing techniques, genetically distinct populations of peregrine falcons can be differentiated thus allowing insights into population structure and dispersal patterns.

· CD spectra of the duplex of $d(AACC)_5 \cdot d(CCTT)_5$ showed that no $C \cdot C^+$ base pairs were present, despite their formation under the same conditions in the individual strands. Thus, in the duplex, $A \cdot T$ base pairs prevent the formation of neighboring internal $C \cdot C^+$ base pairs.

· We developed a technique for producing synchronized populations of human diploid fibroblasts in which 90% of the cells divided within a 3 h period.

· We demonstrated through BrdU-labeling and viable cell sorting techniques that locally developed synchronization procedures will allow us to prepare nearly pure populations of both early- and late-replicating species of DNA from human diploid fibroblasts.

· A synthetic repetitive DNA oligomer that hybridizes specifically to the centromere of human chromosome 18 has been constructed. Chromosome 18 aneuploidy was rapidly detected using this oligomer.

· A consensus alpha-satellite sequence oligomer was synthesized that hybridizes to the centromeres of all human chromosomes. This oligomer was used to rapidly detect chromosome abnormalities, notably dicentrics, following irradiation.

· The human telomere was identified and cloned.

NEURAL PROCESSING AND CHEMISTRY

Project Descriptions:

Neuropsychopharmacology (NIH)

Principal Investigator: J. M. O'Donnell
Co-Investigators: J. F. McElroy, J. J. Stimmel

The goal of this program is to elucidate neuropharmacological mechanisms involved in mediating the actions of drugs used to treat psychiatric illness and drugs of abuse. One aspect of the research investigates how central noradrenergic systems, particularly beta adrenergic receptor subtypes, are regulated and how these systems are involved in mediating the actions of antidepressant drugs. A second aspect of the research focuses on how noradrenergic and dopaminergic systems in the brain are involved in mediating the stimulus and reinforcing effects of stimulant drugs, particularly cocaine. The focus of both research areas is on elucidating basic mechanisms in neurotransmitter receptor regulation that may contribute to the development of pharmacological agents for the treatment of psychiatric illness and drug dependence.

Biological Effects of Microwave Irradiation (DoD)

Principal Investigator: J. M. O'Donnell
Co-Investigators: D. L. Hjeresen, J. C. Fowler, J. F. McElroy, K. O. Umbarger

The goal of this program is to identify behavioral, neurophysiological, and neurochemical effects of microwave irradiation, particulary high power microwave irradiation. In addition, experiments are carried out to identify biological mechanisms that mediate microwave-induced effects. This research contributes to the identification of risks associated with occupational exposure to microwave irradiation.

In Vitro Brain Electrophysiology (DoD, LANL)

Principal Investigator: J. C. Fowler

The overall goal of this research is to determine the identity and actions of endogenous neuroactive substances that are released during metabolic stress in the brain and act to modulate neuronal function. Present experiments involve the characterization of possible endogenous neuromodulators responsible for the depression of neuronal activity during the early period of hypoxia. It is clear that neuronal function is depressed during hypoxia but a major unresolved question is how much of this depression is due to the release of inhibitory substances and how much is a secondary consequence of depletion of energy substrates.

Guanine Nucleotide Metabolism in Rod Photoreceptor (NIH)

Principal Investigator: A. Yamazaki

The objective of this project is to identify biochemical processes for the GTP-dependent signal transduction in rod photoreceptors. Procedures have been developed to purify main protein components for the GTP-dependent cGMP

phosphodiesterase activation. Major unanswered questions are the mechanism of cGMP synthesis (regulatory mechanism of guanylate cyclase) and the functional interaction of guanylate cyclase and cGMP phosphodiesterase.

The Dual Receptor Regulatory Mechanism of Neural Stimulation (LANL)

Principal Investigator: A. Yamazaki
Co-Investigators: F. Hayashi, J. F. McElroy

The goal of this project is to determine how dual receptor systems in neural tissue interact to regulate a common effector pathway. Previous studies of receptor-dependent signal transduction mechanisms have focused on the control of an effector enzyme by an individual receptor type. This study will start in FY 1989 as a new project in LS-1, and will greatly improve our understanding of receptor- and GTP-dependent signal transduction in the brain.

Auditory Neurophysiology (NIH, LANL)

Principal Investigator: D. G. Sinex
Co-Investigators: J. B. Mott and L. P. McDonald

The goal of this project is to provide a functional understanding of the processing of speech sounds by the auditory nervous system. The current experiments examine the representation of selected speech sounds in the responses of primary auditory neurons. The response of the peripheral auditory system is characterized by nonlinear transduction, and by neural discharge rates that saturate at relatively low sound levels but retain fine temporal details that may convey accurate spectral information. Major unanswered questions concern the relative importance of these response properties for the encoding of speech features, and the extent to which the peripheral representation of speech sounds constrains or facilitates the processing of speech by the central nervous system. The results will have implications for reducing speech processing deficits that may accompany hearing loss, or for improving algorithms for computer speech recognition.

Imaging of Neural Activity (LANL)

Principal Investigator: J. S. George
Co-Investigator: J. C. Fowler

The objectives of this project are to investigate patterns of activation in neural networks and to study the cellular mechanisms by which these patterns are learned or modified. The work will utilize a variety of molecular probes (typically absorbance or fluorescence dyes) to monitor the electrophysiological, biochemical and ionic function of intact nerve cells. Low-light electronic imaging techniques will be used to study the kinetics and spatial distribution of neural activity in single cells or in networks of neurons. In the future, we propose to apply these techniques to visualize patterns of activation on exposed cortical surfaces.

Neural Network Model of the Visual Cortex (LANL)

Principal Investigator: J. S. George
Co-Investigator: B. Travis[1]

[1] Earth and Space Sciences Division

This project will model the parallel processing of information by the human visual system. Our computer model employs a biologically accurate model of synaptic function, allows spatial and anatomical patterns of connectivity to be specified, and incorporates a hierarchical structure based on observed structure in sensory cortex. We will use the network to model known physiological function and to explore potential physiological mechanisms for perceptual phenomena such as preattentive texture processing. Such studies should provide insight into biological information processing.

Magnetoencephalography Program (ARI)

Principal Investigator: E. R. Flynn[1]
Co-Investigators: C. Aine, D. Arthur[1], J. George, M. Oakley[1]

[1] Physics Division

Magnetoencephalography involves the application of superconducting sensors to measure the weak magnetic fields associated with electrical activity of neurons in the brain. The long term goal is to develop noninvasive neuromagnetic techniques and procedures for the study, measurement and characterization of human cognitive performance. Our strategy is to proceed in hierarchical fashion beginning with the examination of simple sensory processes (e.g., retinotopic mapping) and gradually adding complexity (associated with higher cognitive functions) to the tasks. Cognitive studies such as a selective attention task typically employ the same stimulus parameters utilized in earlier sensory studies. Models, as they are developed, will be couched in physiological terms to account for the mechanisms underlying such psychological constructs as "attention," "learning," and "memory."

Research Accomplishments:

· The contributions of beta-1 and beta-2 adrenergic receptor subtypes in the brain to behavioral regulation and antidepressent drug activity have been characterized. The results of these experiments support a primary role for the beta-1 subtype in the mediation of antidepressant activity, but support heretofore unsuspected physiological and pharmacological roles for the beta-2 subtype.

· Inhibitors of cyclic AMP phosphodiesterase (PDE) have been found to produce neuropharmacological and behavioral effects similar to those produced by proven antidepressant drugs. The results of experiments completed to date suggest that the cyclic AMP PDE pathway may be a novel system by which pharmacological manipulation can produce antidepressant activity.

· The binding site for cocaine in the brain has begun to be characterized. This binding site, which appears to be complex, may be involved in mediating the stimulus and reinforcing actions of cocaine.

· We have identified thresholds for behavioral disruption produced by exposure to pulses of high power microwaves. This disruption appeared to be related to microwave-induced heating.

· We demonstrated that microwave-induced heating results in adenosine release and depressed neural activity in an *in vitro* brain slice preparation. This release of adenosine may be related to changes in energy balance and may underlie microwave-induced behavioral changes that have been observed.

· We demonstrated that microwave irradiation, even at levels that do not induce heating, reduces noradenergic activity in regions of rat brain. Given norepinephrine's role in behavioral regulation it is possible that this neurochemical change has functional consequences.

· The depression of neuronal activity observed during the first 20 minutes of hypoxia can be substantially blocked by the presence of antagonists of the endogenous neuromodulator, adenosine. This result suggests that adenosine levels rise during hypoxia. Additional experiments show that adenosine levels can be increased even in the absence of synaptic transmission.

· We have continued studies of the cytoplasmic Ca^{2+} transient associated with electrical stimulation of the hippocampal slice. The pharmacological agent APV did not significantly affect the transient, suggesting that the response was not mediated by NMDA synaptic channels. The presence of a response with antidromic stimulation suggests that the response may be mediated by voltage sensitive Ca^{2+} channels in the cell body. Cytoplasmic Ca^{2+} was shown to accumulate if a series of stimuli were presented rapidly, consistent with other observations that a rise in intracellular Ca^{2+} might be involved in the generation of long term potentiation.

· We used purified components of the GTP-dependent signal transduction mechanism in photoreceptors to identify (1) the complex formation of GDP- and GTP-form of $G\alpha$ with inhibitory subunit of cGMP phosphodiesterase, (2) new roles of β, γ subunit of GTP-binding protein in the inactivation of cGMP phosphodiesterase, and (3) new regulatory proteins for guanylate cyclase activation.

· We described the neural encoding of voice-onset time (VOT), the major acoustic feature that underlies the distinction between phonemes such as /d/ and /t/. Both the average rate of discharge and the detailed temporal pattern of response contributed to the neural representation of VOT, for different subsets of neurons.

· A specific neural mechanism that underlies the phonetic categorization of VOT was identified. Statistical variation in the response patterns of primary neurons produces a distorted neural representation of acoustic differences. Good quantitative agreement between this neural representation and the perception of acoustic differences was observed.

· A number of technical improvements have been made to experimental systems for imaging neural activity. Electronic data acquisition systems for single channel measurements and imaging systems have been assembled, and software systems for acquisition and analysis have been developed.

· The primary code that solves the neural network model has been completed, tested, and debugged. An improved model of the retina is now nearing completion. The model takes into account the continuously graded responses and direct electrical interconnections of photoreceptors. It also attempts to incorporate a more accurate description of properties of retinal interneurons.

· We have generated and tested model responses to simple stimuli such as a moving luminance boundary and oriented bars. We have designed software to generate a range of more complex stimuli. A number of graphic tools have been developed for displaying aspects of model function, including 3-D images of the structure of individual neural types, pseudocolor or contour maps of patterns of connectivity of activation, waveforms, spike frequency histograms, and color stripe maps.

· We have developed a video-based system for rapidly digitizing and displaying the head surface for MEG studies. The system produces a plane of light which intersects the subject's head; the resulting bright contour is viewed by an off-angle video camera. The series of thresholded video images are subject to a series of coordinate transforms to produce the surface model. The system is used to document head geometry, and to locate anatomical or instrumental references. Pseudocolor maps representing measured neuromagnetic field distribution can be displayed on the head surface model.

· We have developed software systems to produce and manipulate a 3-D volumetric model from a series of magnetic resonance image (MRI) slices. The model structure allows MRI and neuromagnetic coordinate systems to be reconciled so that the anatomical source of a neuromagnetic field distribution can be identified. The structure is also suitable for future extensions such as magnetic source modeling and "conformal" mapping of the cortical surface.

· We measured neuromagnetic field distributions associated with spatial frequency manipulations. Differences in response latency, in the number and relative strength of response field extrema, and/or shifts in the location or orientation of apparent neural sources were seen. Manipulation of grating contrast produced response differences that were most apparent for the lower spatial frequencies. The results may reflect differences in the relative activation of magno- and parvocellular pathways.

· We demonstrated a strong correlation in waveform morphology for simultaneous electrical and magnetic recordings. These results suggest that some electrical/neuromagnetic "components" appear to reflect focal activation of discrete cortical loci; however, other "components" may reflect extremes of a spatial-temporal path of cortical activation. Computer modeling experiments aid in differentiating between competing source models.

MOLECULAR STRUCTURE

Project Descriptions:

Biological Neutron Scattering (DOE/OHER, LANL)

Principal Investigator: J. Trewhella
Co-Investigators: T. Sosnick, S.E. Rokop, J. Hammer, P.A. Seeger[1]

[1] Los Alamos Neutron Scattering Center

This project encompasses a number of sub-projects whose linking thread is the use of neutron scattering techniques to understand biological structure and function.

Chromatin Studies (with E.M. Bradbury)
There are abundant structural data available concerning the structure of the inactive nucleosome core particle, the smallest and most stable chromatin subunit. However, little is known concerning the details of the structure of the DNA that links the core particles, or about the structures of chromatin subunits in different states of activation. Experiments are in progress to address these issues, with the long term goal to be able to understand the structural basis for processes such as translation and transcription of the genetic code.

Antibody/Antigen Studies (with J. Novotny, U. of Virginia, and D. Benjamin, Squibb Institute)
While there are large amounts of structural data on antibody fragments and on hinge deletion IgG's, relatively little is understood of the complete native structures in solution. By labelling the antigen binding sites with deuterated antigen, the distance between the antigen binding sites can be determined for each subclass of a specific monoclonal IgG using neutron scattering. Such data will provide a basis for understanding the solution structures of this major class of immunoglobulins, the functional significance of the different subclasses, and their role in triggering an immune response.

Membrane Protein Structure (with B. Cornell, CSIRO, Australia)
The objective of these studies is to expand the structural data base on membrane spanning proteins - an important but relatively unknown class of proteins - from the structural perspective. Fourier transform infra-red and nuclear magnetic resonance spectroscopies are being used to assess internal secondary structure content of the membrane protein bacteriorhodopsin. These data may be used to derive models for the structure that can be tested against neutron diffraction data from deuterium labelled bacteriorhodopsin in order to fit the amino acid sequence into the low resolution structure derived from electron microscopy. A detailed structural model of bacteriorhodopsin will provide the basis for understanding the molecular mechanism of its function in the conversion of light energy to chemical energy for metabolism.

Structural Changes and Signalling in Calcium Regulators (NIH, LANL)

Principal Investigator: J. Trewhella
Co-Investigators: D. Heidorn, S. Henderson, S. E. Rokop, R. Rusnack, J. Hammer, P. Seeger[1], D. Walsh[2], A. Means[3], D. Blumenthal[4], H. Crespi[5].

[1]Los Alamos Neutron Scattering Center, [2]University of California, [3]Baylor College of Medicine, Texas, [4]University of Texas Health Center at Tyler, [5]Argonne National Laboratory.

The objective of this project is to understand the underlying molecular mechanism for regulation of biochemical processes by calcium, the major regulator of intra-cellular processes on short time scales. Neutron and X-ray scattering and Fourier transform infra-red spectroscopy are utilized to study the structures of calcium regulating proteins and their interactions with target enzymes in different functional states. The neutron studies utilize deuterium labelling of specific proteins and solvent matching techniques to study individual components in interacting assemblies. Deuterated proteins are produced using bacterial expression systems for cloned sequences. Target enzyme binding fragments are produced by peptide synthesis. Neutron resonance scattering will be used to measure the distances between calcium binding sites within individual molecules. Fourier transform infra-red spectroscopy is used to evaluate internal secondary structural compositions and changes with functional state. Our initial focus has been on the calcium binding protein calmodulin and its interactions with two target enzymes from muscle cells that are activated in calcium dependent processes: myosin light chain kinase and phosphorylase kinase. Some work has also been done on the evolutionarily related calcium binding protein troponin C. The effects of calmodulin glycation have been studied as part of a project that concerns the histopathology of diabetes. Future studies will focus on more fully characterizing the conformational changes induced by calcium binding in these systems in order to understand the underlying molecular mechanisms of calcium dependent enzyme activation.

Renal Handling of Glucoalbumin in Diabetes and Aging (NIH, LANL)

Principal Investigator: A. Kowluru
Co-Investigators: R. Kowluru, M. W. Bitensky

The objective is to understand the mechanisms underlying the loss of editing of glucoalbumin in diabetes and aging and also the molecular basis for increased glomerular filtration rates seen in diabetes. The mammalian nephron selectively excretes glucoalbumin. This phenomenon that is called "editing" is lost gradually in aging and abruptly in diabetes. The loss of editing observed in diabetes is accompanied by increases in glomerular filtration rate and microalbuminuria. In contrast, attenuation of editing in aging is unaccompanied by any of the above. We are proposing that in the aging glomerulus, the net negative charge is increased possibly due to increased synthesis of glucosaminoglycans and sialic acid-containing macromolecules.

Red Cell Na^+ Pump in Diabetes (LANL)

Principal Investigator: R. Kowluru
Co-Investigators: A. Kowluru, M. W. Bitensky

We are studying the molecular basis of the Na^+ pump lesion and its relationship to sorbitol production, myoinositol metabolism, and increased cell volume in diabetic red blood cells (RBC). In particular, increased volume of RBC could pose serious problems in microcirculation generally encountered in long-standing diabetes. These studies have profound significance in understanding the molecular causes of red cell abnormalities and the basis for the catastrophic retinal and glomerular complications that appear in association with, and as a consequence of, the diabetic microangiopathy.

Analysis of Protein Mixtures (LANL)

Principal Investigator: A. G. Saponara
Co-Investigator: E. B. Shera[1]

[1] Physics Division

Complex mixtures of proteins are readily resolved into their constituents by gel electrophoresis in two dimensions. Methods for the detection of proteins on gels are far less satisfactory especially when the proteins of interest are not radioactively labelled. Our objective is to develop methods to directly visualize and quantitate gel patterns. Radioactive two dimensional protein gels are routinely analyzed, quantitatively and with rapidity, by means of a multiwire proportional counter and data acquisition system. We are currently developing methodologies and instrumentation that will allow fluorescently stained gels to be analyzed with similar facility.

NMR Spectroscopy and Imaging of Multicellular Spheroids (NCI,DOE)

Principal Investigator: J. P. Freyer
Co-Investigator: L. O. Sillerud

The objective of this project is to apply NMR spectroscopy and imaging techniques to the study of energy metabolism of tumor cells in a nutrient stressed environment. We are approaching this investigation from two directions: (1) ^{31}P NMR spectroscopy of bulk samples of intact spheroids; and (2) proton and chemical-shift imaging of conditions inside single, intact spheroids. We have developed methods for performing spectroscopy while the spheroids are perfused with medium under conditions exactly matching those used during normal spheroid culture; a similar system for use during NMR imaging is under development. Initial spectroscopy work has focused on characterizing the metabolic and microenvironmental status of spheroids as a function of growth and necrosis development. We have also begun studies of the effects of radiation on energy metabolism in spheroids. The initial imaging work has focused on obtaining high-resolution proton images of slices through intact spheroids. Future efforts will revolve around addressing specific questions about the regulation of tumor cell energy metabolism through altering the nutrients in the medium during spectroscopic analysis and by obtaining images of metabolite concentrations inside the spheroid rim. This system should prove extremely valuable in understanding the complex relationships between cellular energy metabolism, the local cellular microenvironment, and the maintenance of cellular viability.

Metabolism of Cell Walls from Monocotyledenous Plants: ^{13}C Metabolic CNMR Metabolic Analysis of Cell Walls Isolated from Proso Millet ("Panicum miliaceum" L. cv. Abarr) (DOE/BES)

Principal Investigator: L. O. Sillerud
Co-Investigator: N. Fink

The objective is to characterize the structure and dynamics of plant cell wall polysaccharides with the aid of high-resolution ^{13}C NMR spectroscopy. The metabolism of glucuronoarabinoxylans from the *Panicum miliaceum* cell wall is being examined in cells grown on [1-^{13}C]- and [2-^{13}C]-glucose. The low natural-abundance of ^{13}C (1.1%) is advantageous in metabolic NMR studies that follow the fate of specifically labeled ^{13}C precursors because of the low natural abundance background signals. In addition,

the metabolism of the cell wall *in vivo* as it occurs in a perfusion system that measures oxygen consumption, pH and temperature during the course of the NMR experiments is monitored.

Diagnosis of Prostate Cancer with NMR (NIH/ACS)

Principal Investigator: K. R. Halliday[1]
Co-Investigators: L. O. Sillerud, R. H. Griffey,[1] J. Hutson, E. Clinard

[1] University of New Mexico, Albuquerque

^{13}C nuclear magnetic resonance (NMR) spectroscopy is being used to distinguish human prostatic cancer from other, benign diseases of the prostate. The method is based on the observation that the normal, non-malignant prostate contains very high concentrations of citric acid and that the amount of this tissue metabolite decreases markedly during the process of neoplastic transformation. Natural abundance ^{13}C NMR spectroscopy is used to non-invasively monitor the amounts of citrate in prostate tissue. The long-term goal is to develop methods that will enable the distribution of citrate to be imaged *in situ* for clinical applications. This will be achieved with the aid of new NMR metabolic imaging techniques.

Applications of Stable Isotopes (DOE/OHER)

Principal Investigator: L. O. Sillerud
Co-Investigator: N. Fink

This project is devoted to the development of non-invasive NMR methods for the detection of added stable isotopes in biomolecules for the elucidation of metabolism and structure of biomolecules. Our research has emphasized various investigations as follows: tumor metabolism, gut ischemia, DNA structure and dynamics, and biocalcification.

Tumor metabolism. The normal prostate maintains high amounts of citric acid that are secreted into seminal fluid. Prostate tumors, on the other hand, metabolize citrate into cellular lipids. We are seeking to utilize ^{13}C-labeled glucose to monitor the metabolism of citrate in cultured prostatic tumor cells with the aid of ^{13}C NMR.

Gut Ischemia. Intestinal ischemia is a significant source of mortality in cases of cardiac arrest and resuscitation. An important medical procedure would be one that would be able to measure the presence and extent of ischemic gut in real time, without surgical intervention. Phosphorus-31 NMR spectroscopy is capable of monitoring tissue energy metabolism.

DNA Structure and Dynamics. The structure and mobility of DNA in solution are parameters that determine many important properties of this biopolymer. All of the bases contain nitrogen that can be biosynthetically labeled with the magnetic nucleus ^{15}N. The labeling and isolation of large quantities of ^{15}N-DNA have paved the way for detailed investigations of the magnetic ring-current shifts due to base-stacking and base-pairing in single- and double-stranded DNA.

Biocalcification. ^{13}C NMR has been applied to determine the binding of calcium to peptides containing the new amino acid, aminomalonic acid.

Research Accomplishments:

· X-ray studies have revealed that the solution and crystal structures of the calcium binding proteins calmodulin and troponin C differ in significant ways in regions thought to be associated with target enzyme binding.

· Fourier transform infrared spectroscopic studies have revealed details of the changes in secondary structure induced by calcium binding to calmodulin.

· Neutron and X-ray scattering studies have shown that large conformational changes are induced in calmodulin by target enzyme binding.

· Details of the mechanism for functional impairment of calmodulin caused by glycation have been revealed using X-ray scattering and circular dichroism.

· Reconstituted nucleosomes with fixed lengths of DNA have been made and neutron scattering data measured in order to assess the path of the DNA as it enters and leaves the core nucleosome particle.

· Bacteriorhodopsin labelled with C^{13} amino acid labels has been made and reconstituted into lipid vesicles for NMR studies of the secondary structure in the membrane spanning portions of this protein.

· Deuterated nutrients have been isolated for use in obtaining deuterated proteins in pure form from bacterial expression systems. These systems are used for obtaining deuterated antigens of other protein components in neutron scattering experiments.

· Proteins can be readily labelled with radioactive rare earths to high specific activity by the simple expedient of brief incubation at room temperature. We are currently attempting to adopt this procedure to detect and quantitate proteins following their separation by gel electrophoresis.

· Na^+ pump activity in diabetic red cells showed a decline that was strongly correlated with increases in cell volume and degree of hyperglycemia. In addition, significant changes in the rates of filtration of diabetic RBC through a 4.7μ nucleopore filter were observed.

· Preliminary data indicate that Na^+ pump activity is regulated by phosphorylation-dephosphorylation reactions in red cells and is mediated by C-kinase. We plan to extend these observations to study the regulation of Na^+ -K^+ ATPase activity in red cells by C-kinase agonists and also by myoinositol, a precursor for DAG synthesis.

· The fractional clearance of dextrans (22°A) with varying charge was studied in young and old rats. Preliminary data suggest that the glomerular filter becomes increasingly negative as a function of age.

· A perfusion system has been developed and tested for maintaining large numbers (up to 5 x 10^8 total cells) of intact spheroids in suspension culture while confined in the NMR magnet for spectroscopic analysis. This system incorporates continuous monitoring of the medium pH, temperature, and oxygen content. Studies have shown that spheroids grow in this system as well as in normal spinner flask culture, and can be maintained in the NMR instrument for several days.

· Analyses of ^{31}P spectra of spheroids of two cell lines as a function of spheroid size have demonstrated that as spheroids grow and necrosis develops, the mean pH drops almost 1 unit and the P_i:NTP ratio increases almost 10-fold. These results are similar to those found with tumors *in vivo*, and suggest that changes in tumor cell metabolism and in the local cellular microenvironment account for the observed effects *in vivo*, as opposed to host physiological alterations.

· Preliminary studies have shown that energy metabolism and pH improve in spheroids within 24 hours of a dose of radiation sufficient to cause clonogenic death in 90% of the tumor cells.

· High resolution (~10 μm) images of sections through an intact spheroid have been obtained. The areas of viable cells and central necrosis can be easily distinguished due to the relatively high relaxation time (T_2) in the viable rim area.

· The relative rates of the pathways that contribute carbon atoms to the residues in the *Panicum* cell walls have been measured using ^{13}C NMR spectroscopy.

· ^{13}C NMR spectroscopy has been used to measure citrate levels in rat prostate tissue *in vitro* and in human prostate tissue *in vivo*. The citrate levels have been observed to decrease in prostate tumors, as expected.

· Phosphorus-31 NMR spectroscopy has been established as useful in determining the presence and extent of intestinal ischemia. A classification scheme has been developed for differentiating ischemic gut from healthy tissue based on the ^{31}P spectrum of the abdomen.

· ^{15}N NMR spectroscopy has been used to establish that the nucleotide bases in single- and double-stranded DNA undergo rapid, isotropic reorientation on the nanosecond time scale.

FLOW CYTOMETRY

Project Descriptions:

High Speed Single Cell Spectrometry (LANL)

Principal Investigator: T. N. Buican
Co-Investigator: J. C. Martin

Our objective is to develop a high-performance flow cytometer, capable of analyzing the emission spectra of individual cells. A proof-of-principle version of the instrument, FTCS-1, has been completed. We are currently designing a more sensitive version that could be used as an attachment on conventional flow cytometers.

Single Cell Analysis and Manipulation by Optical Trapping (LANL)

Principal Investigator: T. N. Buican
Co-Investigators: J. C. Martin

We developed cell and chromosome manipulation techniques based on the radiation forces exerted by collimated laser beams (optical trapping). These techniques have the positional precision of mechanical micromanipulators, do not require any significant fluid flow or mechanical contact with the biological particles, and can operate inside fully enclosed chambers. We also developed a prototype instrument capable of imaging and manipulating cells and chromosomes, which was awarded a 1988 R&D-100 award. The instrument will be particularly useful in the study of cell-cell and cell-substrate interactions, as well as in controlled cell fusion and transfection.

Conceptual Design for an Advanced Cytology Workstation for the Space Station (NASA)

Principal Investigator: T. N. Buican

We developed a conceptual design for an advanced instrument to be used on the Space Station Life Sciences Research Facility. The instrument, the design of which is based on that of our optical trapping cell manipulator, will be capable of performing complex analytical and preparative procedures in a space-based biology laboratory, and could also have important applications in terrestrial laboratories.

An Advanced Optical Manipulation Chamber (NASA)

Principal Investigator: T.N. Buican
Co-Investigator: B. D. Upham

We are developing an advanced optical manipulation chamber system that will allow complex, long-term biological experiments to be performed by an optical trapping workstation. The new chamber contains multiple microscopic compartments for the examination and separation of cells and chromosomes. Furthermore, the compartments are connected with each other and with the outside through microscopic channels, through which single biological particles can be transported and reagents and media can be circulated. The temperature of the manipulation chamber can be accurately

controlled. We are also developing machine vision software that will allow the optical trapping workstation to analyze and manipulate biological particles without operator intervention.

National Flow Cytometry Resource (NIH)

Principal Investigator: L. S. Cram
Co-Investigators: M. F. Bartholdi, R. Beckman, T. Bulcan, H.A. Crissman, J. Jett, J. Martin, G. Salzman, J. Steinkamp, C. Stewart, M. Wilder, R. Habbersett, M. Cassidy, J. Parson, K. Albright, J. Fawcett, R. Hiebert

The focus of the National Flow Cytometry Resource is in five areas: technological research and development, collaborative research projects, service projects, training, and dissemination. During the fifth year of the grant period, the Resource was involved in a number of projects. Though too numerous to list in their entirety, the following projects are examples of collaborations the Resource undertakes.

Identification of Pulmonary Macrophages by Flow Cytometry. Interstitial pulmonary macrophages have been identified and separated using the Multiparameter Flow Cytometer. Subpopulations are identified by differential fluorochrome-labeled lectin binding, differences in electro-optical phenotype, and flow cytoenzymatic analyses. Following sorting, the cells undergo examination of their ultrastructural characteristics (electron microscopy) and basic macrophage functional activities.

Identification of Robertsonian Mouse Chromosome by Flow Cytometry. The Robertsonian mouse chromosome 4.11 has been cleanly resolved from other chromosomes and background debris by bivariate flow karyotyping. Chromosomes are being sorted for construction of a recombinant DNA library.

Supernumerary Marker Chromosomes. Marker chromosomes of unknown origin that are found in children's disorders are being sorted. Hybridization techniques will be used to identify the constitution of the markers. Deleted Y chromosomes from a patient have been sorted for molecular identification.

A Zeeman Interferometer Air Monitor (ACRDEC)

Principal Investigator: R. G. Johnston

The advent of the two-frequency Zeeman effect laser greatly simplifies interferometry. Using this new kind of laser, we are developing a simple "Zeeman interferometer" to monitor air or water for the presence of contamination based on a change in the air's bulk refractive index, n. By focusing the beam from the Zeeman laser, we hope to be able to count and "weigh" individual particles in air (or water) based on their optical retardation. Applications to be explored include: air or water quality monitoring, flow cytometry, and ultrasensitive detectors for spectrophotometry, CD, LD, chromatography, and gel electrophoresis.

Phase-Resolved Measurement of Fluorescence Lifetimes (DOE, NFCR)

Principal Investigator: J. A. Steinkamp
Co-Investigators: J. C. Martin, B. F. Bentley, H. A. Crissman

Phase-resolved measurements of fluorescence lifetimes on single cells are under development. The goals are to develop the technology for making phase-sensitive measurement of fluorescence lifetimes on cells stained with fluorochromes as they intersect a modulated laser excitation source; to use fluorescence lifetime as a parameter for studying fluorochrome interactions with cell constituents; to be able to resolve the fluorescence signals from dyes having overlapping emission spectra,

but different lifetimes; and to investigate ways for improving signal-to-background noise. Stained cells are analyzed as they flow through a chamber and intersect a modulated (sinusoid) laser beam. The resulting fluorescence signals, which are modulated and shifted in phase from the laser excitation (reference), are processed by phase-sensitive detection electronics for phase and amplitude quantification. The phase-sensitive detection method is presently being evaluated to determine the "best approach" for quantifying phase shift information from signals having nanosecond lifetimes using analog, digital, or hybrid electronics. In addition, a mathematical model is being developed for describing the fluorescence signal as a function of the phase detection method. The model will be used to develop a clear understanding of and to optimize flow cytometric phase-resolved measurement of fluorescence lifetimes.

Combination Detection System (DoD)

Principal Investigator: G. C. Saunders
Co-Investigators: J. C. Martin, J. H. Jett, M. E. Wilder, B. F. Bentley, J. Lopez, L. Hutson, E. Martinez

The objective of this program is to develop techniques and the necessary instrumentation for the near-real-time detection of either chemical nerve or biological agents present in air and/or water. Nerve agent detection is based on acetylcholinesterase activity in the presence of a fluorogenic substrate; nerve agents poison the enzyme resulting in a decrease of fluorescent product production. Bioagent detection is based on the fact that most live agents and toxins will have associated with them high levels of extraneous proteins and amino acids. Using appropriate fluorophors, the instrument will continuously monitor the environment for the presence of amino acids and proteins. A sudden increase above background would signal the possible presence of bio/toxin agents. Our goal is to develop a compact, robust instrument capable of simultaneous detection of nerve and bioagents.

Light Scattering from Biological Cells (NIH, ACRDEC)

Principal Investigator: G. C. Salzman
Co-Investigators: R. G. Johnston, S. B. Singham, W. K. Grace, C. F. Bohren

The goal of this program is to determine what can be learned about biological cells with polarized laser light scattering. The coupled dipole method is being used to build theoretical models of a variety of bacteria. An arbitrary particle is subdivided into units each smaller than the wavelength of light. A unit is assumed to behave like a spherical dipole oscillator with its polarizability specified by its size and the bulk dielectric constant of the particle. We are measuring the polarized light scattering properties of homogeneous suspensions of bacteria and comparing the experimental data with the theoretical models. The measurements are made using a Zeeman effect laser.

Ultraviolet Fluorescence LIDAR (ACRDEC)

Principal Investigator: G. C. Salzman
Co-Investigator: R. R. Karl[1]

[1] Earth and Space Sciences Division

The goal of this project is to develop a transportable LIDAR (Light Detecting and Ranging) system for remotely detecting the presence of biological material in an aerosol cloud. A krypton-fluoride pulsed laser excites fluorescence from the

aromatic amino acids present in the biological material. A 40 cm diameter telescope captures fluorescence emission from the aerosol.

Novel Fluorescent Probes for Flow Cytometry (LANL)

Principal Investigator: B. L. Marrone
Co-Investigators: T. W. Whaley, C. J. Unkefer[1]

[1] Isotope and Nuclear Chemistry Division

The scope of this new proposal is the design, synthesis and application of fluorescent probes for (1) single cell analysis of enzyme activity by flow cytometry, and (2) chromosome mapping. Our initial effort will be concentrated on probe development for single cell analysis of enzyme activity. The need for a system with which to investigate enzyme activity on a single cell basis grew out of ongoing research on factors regulating the growth and differentiated function of ovarian cells. Being secretory cells, ovarian cells are identified best by their metabolic products, which result from the cell's hormone receptor-mediated, enzyme activities. Current single cell analysis techniques, such as flow cytometry, have limited usefulness for the study of cell function and metabolism since probes are not available that would capture the relevant cell enzyme activities. We envision that the methodologies and strategies that we develop to probe the metabolism of ovarian cells will be generally useful for studies in which the desired endpoint involves measurement of function and metabolism in single cells from heterogeneous cell populations.

Advanced Flow Cytometric Technology (DOE)

Principal Investigator: J. H. Jett
Co-Investigators: J. C. Martin, M. E. Wilder, T. N. Buican

This program provides the base instrumental support for the flow cytometry at Los Alamos and contributes to the development of new measurement techniques. Enhancement of the Los Alamos version of the Livermore high speed sorter is continuing. This sorter is capable of making sort decisions at rates of up to 20,000 per second and is being used to sort chromosomes for Phase II of the National Gene Library Project. A MicroVax based computer cluster purchased with an NIH Shared Instrumentation Program grant has been installed and is being used by 50 researchers in Life Sciences Division. It provides them with a large capacity local computing system as well as an access route to the Laboratory Central Computing Facility. Another area of emphasis is sensitive fluorescence techniques which have led to the detection of individual fluorescent molecules. The development of a new generation of data acquisition electronics for flow cytometers and sorters is under way.

Advanced Concepts for Base Sequencing in DNA (DOE,LANL)

Principal Investigators: J. H. Jett, R. A. Keller[1], J. C. Martin, E. B.Shera[2]
Co-Investigators: B. Marrone, R. Ratliff, N. Seitzinger[1], M. Alexander[1], L. Krakowski, J. Hahn[1], R. Moyzis

[1] Chemistry and Laser Sciences Division
[2] Physics Division

New and more rapid techniques for the sequencing of DNA are being investigated. The feasibility of several approaches has been explored in depth including detection

of fluorescently labeled bases in a modified flow cytometer, acoustic microscopy, electron tunneling microscopy, X-ray microscopy, neutron microscopy and mass spectrometry. At the present time, the approach of detecting fluorescently labeled bases in a modified flow cytometer is being pursued most vigorously. The basis of this technique is our projected ability to detect individual fluorescent molecules such as rhodamine as they pass through a laser beam in a modified flow cytometer. By labeling each base in a single strand of DNA, suspending that single strand in the flow system, and sequentially cleaving the end base into the flowing stream, each labeled base will be identified as it passes through the laser beam. Based on the time of detection and the rate at which enzymatic cleavage proceeds, we expect to achieve sequencing rates between 100 and 1000 bases per second.

Research Accomplishments:

· We have completed and delivered to NASA a report entitled "Advanced Cytology Workstation for Space Station: A Conceptual Design."

· We have completed a robotic optical trapping cell manipulator (hardware and software). The instrument received a 1988 R&D-100 award.

· We have demonstrated the optical trapping and manipulation of a variety of cells (erythrocytes, macrophages, thymocytes, plant protoplasts) and of chromosomes.

· Seven major computer programs implementing the coupled dipole model for light scattering from small biological cells have been documented and assembled into a report. The report includes program descriptions, example problems, listings and the software. It is available through NTIS.

· A scanner has been installed on the ultraviolet LIDAR system enabling data collection over an azimuthal angular range of 180° and an elevation range of $\pm$ 15°.

· High-speed (nanosecond) photomultiplier detectors and signal amplifying electronics have been constructed and tested for phase-sensitive detection of fluorescence lifetimes. An electro-optical modulator for modulating the laser excitation intensity has been evaluated and 1 and 10 MHz modulated fluorescence signals have been recorded.

· A novel air sampler which employs a piezoelectric oscillator to generate microdroplets which scrub and concentrate pollutants from incoming air has been designed, built and tested. In conjunction with our pulsed laser fluorimeter, it will detect aerosolized protein at the part per trillion level.

· A complete prototype air/water nerve and bio agent detection system has been built and will shortly be taken "on site" for detailed testing and evaluation.

· As a first step towards preparing DNA strands in which each base is labeled and can be sequenced using a modified flow cytometer, we have replicated 2000 bp strands of DNA from A·G template using biotinylated analogs of dUTP and dCTP and the large fragment of DNA polymerase 1 (Klenow fragment) *E. coli*. The biotinylated DNA is recognized and cleaved by exonucleases in solution.

CELL GROWTH AND DIFFERENTIATION

Project Descriptions:

Utilizing FCM and HPLC to Measure Protein Changes in Isolated Cell Nuclei Resulting from Cell Cycle Progression, Radiation Exposure, and Chemical Treatments (DOE)

Principal Investigator: L. R. Gurley
Co-Investigator: H. A. Crissman

The objective of this program is to identify those constituents and biochemical processes in chromatin that, when altered or damaged, may result in neoplasia. To accomplish this objective, we have developed new analytical methods for flow cytometry (FCM) and high performance liquid chromatography (HPLC) that will permit us to determine how various by-products of energy technology interact with chromatin and disrupt its structural integrity, causing alterations in DNA's normal control of cell proliferation. By developing 3-laser FCM techniques, we can now identify and isolate cells having distinctive alterations in their chromatin structure following exposure to agents such as radiation and toxic chemicals. Then, through utilization of high sensitivity HPLC methods developed in our laboratory we can determine how nuclear protein components have been altered to destroy cell proliferation control. These activities are expected to establish a factual basis for our hypothesis that cell proliferation and metabolism are controlled through modulations of DNA transcription, replication, and segregation brought about by alterations in the complex interactions between DNA and nucleoproteins that are driven by chemical modifications of nucleoproteins such as phosphorylation and acetylation. Results from this program should lead to: (1) identification of specific targets within the nucleus that are sensitive to attack by radiation and toxic chemicals, (2) identification of specific molecular alterations in chromatin structure that are manifested as damaged chromosomes at mitosis, (3) formulation of a model of the biochemical mechanisms of action of γ-rays and chemical toxic agents, and (4) development of new techniques for damage assessment.

Regulation of Cellular Proliferation (DOE, MRDC)

Principal Investigator: W. R. Wharton
Co-Investigators: D. Kim, M. Smyth, B. Stone

The long-term goal of this series of projects is to develop a multilevel understanding of the mechanisms that regulate cell cycle traverse in mammalian fibroblasts. The work entails the investigation of cellular, biochemical and molecular events that occur either as a function of stimulation with mitogenic hormones or as a result of the cell's traverse through a specific portion of the cell cycle. Special emphasis is now being placed on the development of cell cycle mutants with specific lesions in their ability to respond to discrete classes of mitogenic agents. These mutants are being isolated by a combination of standard selection procedures as well as by novel flow cytometric-based, rare event detection protocols.

Growth, Function, and Heterogeneity of Ovarian Cells (NIH)

Principal Investigator: B. L. Marrone

The objective of this project is to understand the mechanisms governing growth and differentiation of steroid hormone-producing ovarian cells during follicular maturation. The animal model used is the domestic hen, a species which ovulates daily. Cells are obtained from individual follicles at well-defined stages of maturation. The approaches used are short-term primary culture of ovarian cells combined with the analytical methods of multiparameter flow cytometry, cell morphometry, and hormone assays. The following specific aims are being addressed: (1) to characterize the granulosa and theca cell populations during follicular maturation in terms of cell cycling, cell size and morphology, cell metabolism, and cell heterogeneity; and (2) to identify the steroidogenic functions of theca and granulosa cell populations and subpopulations, with special consideration of possible cell-cell interactions in the steroidogenic process. Studies during the past year were concentrated primarily on characterizing the regional pattern of cell growth and differentiated function in the granulosa cell layer.

Regulation of Cellular Growth in Multicellular Spheroids (NCI)

Principal Investigator: J.P. Freyer
Co-Investigator: A.G. Saponara

The objective of this project is to use the multicellular spheroid tumor model system to elucidate the mechanisms controlling the growth and viability of tumor cells in the actual *in vivo* tumor. We are approaching this problem from several directions: (1) investigation of the regrowth kinetics of cells after removal from the spheroid microenvironment; (2) characterization of a protein growth inhibitor related to the process of necrosis in spheroids and tumors; (3) elucidation of the mechanism of formation of said growth inhibitor; and (4) analysis of the role of energy metabolism in the onset of necrosis. The results of this project are providing some important clues as to how tumor cells turn on and off the process of cellular proliferation in the actual tumor environment. We are also clarifying the complex relationship between the regulation of cellular viability and proliferation in a nutrient-stressed microenvironment. This project is closely related to our new initiative to use nuclear magnetic resonance techniques to study energy metabolism in spheroids. Other new approaches being investigated are the use of mutant cells deficient in respiration and glycolysis, and the growth of spheroids in a serum-free and growth-factor-free culture medium.

Effect of Radiation on the Immune Response (LANL)

Principal Investigator: C. C. Stewart
Co-Investigator: A. P. Stevenson

The objective of this program is to study the effects of low-dose radiation on the survival and function of lymphoid subsets from human peripheral blood. Monoclonal antibodies directed against leukocyte cell surface markers were used to identify specific subpopulations of lymphocytes and monocytes from blood obtained from laboratory employees who do not work in a radiation environment and from current or past laboratory employees who had depositions of plutonium ranging from 10-100 nCi at the time of blood collection. The most striking finding was that the plutonium workers had significantly elevated CD4 to CD8 ratios (helper T cell/suppressor T cell ratios). This increase was due to a decrease in the absolute number of circulating

$CD8^+$ cells, as $CD4^+$ cell number was normal. When lymphocytes were irradiated *in vitro*, the $CD8^+$ cells were 3 times more radiosensitive than $CD4^+$ cells. These findings, taken together, suggest that a depletion of this subset has occurred over the forty years due to internal radiation.

Research Accomplishments:

· We have developed a flow cytometric method to quantitate adipocyte differentiation based on both fluorescence and light scatter. This system is being used to study the kinetics of the acquisition of various aspects of the fully differentiated phenotype and, using the BrdU technology described elsewhere, to closely investigate the loss of mitogenic responsiveness during terminal differentiation.

· A BALB/c - 3T3 cell selectively deficient in $Na^+K^+Cl^-$ transport was also found to be less responsive than parents to the mitogenic action of insulin-like growth factors. The exact mechanism of the resistance and the nature of the link between transport and receptor activation are presently under investigation.

· Evidence for a regional pattern of differentiation in the granulosa cell layer surrounding the hen oocyte was obtained. Granulosa cells peripheral to the germinal disc region were more steroidogenic and a smaller percentage of them were in S and G2 + M stages of the cell cycle as compared to granulosa cells overlying the germinal disc.

· We have isolated a distinct growth inhibitory protein from extracts of spheroids and tumors with extensive necrosis. This protein is extremely heat and trypsin labile, has a molecular weight of 80-90 kD, and appears to depend on disulfide bonds for activity. This protein is not found in extracts from cells or spheroids without extensive necrosis.

· We have characterized the regrowth kinetics of cells after separation from different regions within spheroids for four different cell lines. Proliferating spheroid-associated cells resumed growth with no lag time, while quiescent cells from spheroids required 1.5-2 times the normal cell cycle transit period to resume growth. The majority of spheroid quiescent cells are in the G_1-phase, and most of the growth lag period is spent in this cell cycle phase.

· We have developed a unique flow cytometric method that allows the resolution of eight human leukocyte subsets in addition to dead cells. The technique requires two-color excitation, detection of four colors of emission, and the use of six monoclonal antibodies simultaneously with propidium iodide.

RADIATION BIOLOGY

Project Descriptions:

Molecular Mechanisms of Radiation-Induced Mutations in Human Cells (DOE/OHER)

Principal Investigator: D. J. Chen
Co-Investigator: R. T. Okinaka

The objective of this program is to study the mechanistic basis for gene mutation induced by ionizing-radiation in normal human fibroblasts. Special emphasis is on high LET radiations such as α-particles and heavy ions. Specific aims are (1) determination of the mutation-induction frequencies as a function of LET [10-2000 KeV/μm]; (2) molecular analysis of the type and extent of DNA damage at the HGPRT locus by Southern blotting techniques; and (3) characterization of the size of deletion by pulse field gel electrophoresis. The results of this study will contribute to a better understanding of the molecular mechanism of high-LET radiation-induced mutations in normal human cells and will test the hypothesis that high LET-induced mutation is primarily due to deletion and that the size of deletion depends largely on the quality of the radiation.

Cytogenetic Effects Pertaining to Low Doses of Radiation (NCI)

Principal Investigator: M. N. Cornforth

The objective of this research is to obtain information concerning mechanisms involved in the production and subsequent modification (repair) of radiogenic lesions responsible for chromosomal aberrations. Emphasis is placed on the testing of various models of radiation action in the context of their ability to accurately predict cellular response to extremely low levels of ionizing radiation. More specifically, experiments have been designed to test the ability of so-called "repair-saturation" models of radiation action to explain various aspects of exchange aberration formation. This is being accomplished using ultra-low energy X-rays (< 0.3 KeV) coupled with the technique of premature chromosome condensation (PCC), as well as cell fusion (between irradiated and unirradiated cells).

Chromosome Damage in the One Rad Region: Cytogenetic Detection Following Ultra-Low Doses and Dose Rates (DOE/OHER)

Principal Investigator: M. N. Cornforth

The overall objective of this proposal is to provide quantitative data concerning both the dose-response and repairability of cytogenetic damage caused by ultra-low doses of densely ionizing radiation. It is felt that this represents a direct and scientifically sound approach to the problem of radiological risk assessment in human populations that are exposed to *realistic* levels of radon, and radon daughter products. Basic aims of the research are (1) to measure the radiation-induced breaks that are produced in prematurely condensed chromosomes (PCC) of normal human cells, following acute doses of 5 MeV alpha particles ranging from 1 to 10 rad; (2) *in situ* hybridization of cloned repetitive DNA fragments to identify centromeres in prematurely condensed chromosomes (PCC) of normal human cells. This improvement in

methodology should extend the usefulness of PCC analysis in a number of ways, including (a) ability to quantitate the incidence of misrepair at the cytogenetic level, (b) increasing the resolving power of the system, allowing damage caused by even lower doses to be detected, (c) long-term potential for automated analysis of radiation-induced chromosomal aberrations; and (d) correlation of the frequencies of radiation-induced aberrations in PCC and metaphase chromosomes to cell killing at very low dose rates, to determine a relative biological effectiveness (RBE) for 5 MeV a particles that is valid for situations of extremely small absorbed dose. Although this is a relatively new program, and much of the effort to date has been spent on the construction and dosimetric calibration of the ^{238}Pu source to be used, preliminary results suggest the following: (1) alpha emissions, as measured by breaks in PCC, are approximately twice as effective as γ rays per unit dose in causing initial damage to chromatin. Also, for a given level of initial damage, α-induced breaks are less effectively repaired; and (2) the stochastic nature of damage produced by α particles becomes a significant consideration, even at relatively high doses. Consequently, it may not be necessary to directly measure damage to cells at doses as low as originally thought (i.e. ~1 rad) in order to extrapolate measured responses down to those associated with environmental levels of exposure.

Chelation of $^{239}Pu^{4+}$ by a Siderophore (DOE, LANL)

Principal Investigator: L. E. Hersman

Siderophores are described as low-molecular-weight (500-1000 daltons) virtually ferric-specific ligands, the biosynthesis of which is carefully regulated by iron and the function of which is to supply iron to the cell. It is possible that Pu^{4+} may serve as an analog to Fe^{3+} because of the similarity in their charge/ionic-radius ratio (4.6 and 4.2, respectively). The purpose of this project is to determine the role that siderophores play in the transport of actinide elements away from the proposed high level nuclear repository, and to describe the nature of the siderophore/actinide complex. This work will be applied to the development of models designed to predict the movement of actinide elements through the environment, and to developing a better understanding of the structures of organo/actinide complexes.

Bioreclamation of Toxic Wastes (LANL)

Principal Investigator: L. E. Hersman

The objective of this project is to investigate the potential use of indigenous soil microorganisms for the *in situ* bioreclamation of toxic wastes. The major contaminants found in the groundwaters of the U.S. are halogenated aliphatic hydrocarbons (e.g., chloroform, trichloroethane, trichloroethylene, tetrachloroethylene, and carbontetrachloride). Recent evidence in the literature suggests that methane oxidizing microorganisms are able to dehalogenate the compounds. It is believed that monooxygenase enzymes produced by the methane oxidizing bacteria epoxidize these coumpounds. The applications of these studies are very important in that by using natural gas (methane) it may be possible to stimulate indigenous, methane oxidizing microorganisms to degrade halogenated, aliphatic toxic wastes. We are currently investigating the biodegradation of tricholoroethylene (TCE) by several methane oxidizing bacteria.

Cellular Radiobiology (DOE, NCI)

Principal Investigator: M. R. Raju
Co-Investigators: M. N. Cornforth, J. P. Freyer, D. T. Goodhead[1], and M. E. Schillaci[2]

[1] Medical Research Council, Chilton, United Kingdom
[2] Medium Energy Physics Division

This effort consists of three interrelated projects which utilize common radiation sources, dosimetry, cell lines, and flow cytometry instrumentation. Advances in each area complement the efforts in the other projects and serve to better define the research goals and experimental techniques used to approach these goals.

A. Radiobiology of Ultrasoft X-rays (DOE/OHER)

Ultrasoft x-rays (<5 KeV) produce biological lesions at the nanometer scale and are thus a good probe to study the physical and biological parameters associated with such lesions. Results of biological experiments with ultrasoft x-rays can also be used to directly test models of radiation action on biological systems. Los Alamos National Laboratory has highly developed ultrasoft x-ray sources and calibration facilities. We are using these unique resources together with our flow cytometry instrumentation and expertise to study the effects of ultrasoft x-rays on mammalian cells. Our results to date show that the relative biological effectiveness (RBE) of ultrasoft x-rays for cell killing depends strongly on how the dose to the nucleus is calculated. When the dose is linearly-averaged over the entire cell nucleus, the RBE decreases with decreasing nuclear thickness. This result holds both for different cell lines with various nuclear configurations and for mutants of one cell line having differing nuclear thicknesses. We are developing a dosimetry model which takes into account the non-linear nature of cell survival with radiation dose. Future efforts will focus on chromosome damage, mutation, and transformation studies with these x-rays.

B. Radiobiology of α-Particles from Plutonium (DOE/OHER)

The initial studies of cell killing by α-particles suggested that the traversal of a single particle through the nucleus is sufficient to inactivate the cell. The current literature demonstrates a wide range in the number of α-particle passages required to inactivate a cell (up to 20). This variation may be due to differences in the geometry of the cell nucleus at the time of exposure. We are investigating this problem using different cell lines with various nuclear cross-sectional areas, as well as using the same cell line under different attachment conditions. We have also developed an α-particle source with which we can vary the energy of the particles incident on the cell nucleus. Since the attenuation of the α-particles is dependant on their energy, we can use this source to expose the entire cell nucleus, a portion of the nucleus, or only the nuclear membrane region. Future work will concentrate on cell survival measurements after exposing different nuclear regions of cells with different nuclear configurations.

C. Age Response and Oxygen Enhancement Ratio (OER) Measurements Using Flow Cytometry (NCI)

In spite of many years of effort, the following basic radiobiological questions are still not resolved: (1) Is the oxygen effect a pure dose modifying factor, or does its magnitude change with radiation dose? (2) Does the oxygen effect change with position in the cell cycle? (3) Are cells with a short G_1-phase radioresistant only in S-phase, unlike cells with a long G_1-phase (which are also resistant in very early

G_1)? and (4) Does the reduction in radiosensitivity as a function of cell-cycle position for high-LET radiations depend on cell type? The objective of this project is to take advantage of the flow cytometric tools available at Los Alamos to address these unresolved problems using several cell lines but a common experimental approach. Our newly developed volume cell sorter has the capability of reproducibly plating known numbers of cells for precise low-dose survival measurements, and can also separate cell-age compartments from an asynchronously growing cell population. We have found no statistically significant variation in OER as a function of dose, although the use of certain common radiation survival models do predict such a variation. Future work will concentrate on studies of OER as a function of cell-cycle position, using sorted populations and cell lines with different G_1-phase durations. We will also compare OER as a function of cell cycle position after exposure to gamma-rays and α-particles.

Radiation Damage of Eukaryotic DNA and Its Repair (NCI)

Principal Investigator: R. J. Reynolds
Co-Investigator: R. L. Ratliff

The goals of this project are to examine the induction of closely opposed pyrimidine dimers, the mechanisms by which they are repaired and the consequences of their induction in eukaryotic cells. It is believed that the bifilar nature of closely opposed dimers makes their elimination by normal cellular DNA repair mechanisms more complicated and that their induction will therefore be of greater biologic significance than the induction of dimers at relatively isolated positions. Sensitive enzymatic assays developed in this laboratory have facilitated the detection and quantification of closely opposed dimers and have been used to partially characterize the cellular functions necessary for the initiation of their repair. Current studies are concerned with the replication of DNA past closely opposed dimers, the effects of DNA sequence on the induction of closely opposed dimers, and the role of closely opposed dimers in the induction of genetic recombination by UV radiation. Throughout these studies, results with isolated and closely opposed dimers are being compared to determine the relative importance of unifilar and bifilar DNA alterations.

Mechanisms of Radiation and Chemical Genetic Toxicology (DOE/OHER)

Principal Investigator: G. F. Strniste
Co-Investigators: D. J. Chen, M. A. MacInnes, R. T. Okinaka, R. J. Reynolds

The objective of this task is to elucidate molecular mechanisms mediating the induction of heritable change by radiation and environmental chemicals. This objective is being pursued through the characterization of DNA structural alterations and the clarification of cellular repair processes that act on these lesions. A major focus of this effort has been the isolation and characterization of human DNA repair genes. Various experimental approaches employed include (1) chromatographic, electrophoretic (including pulsed transverse alternating field electrophoresis), and sedimentation procedures for the qualification and quantification of DNA lesions; (2) Southern blot analysis and polymerase chain reaction (PCR) amplification for the detection of DNA structural change at specific genetic loci; (3) complementation of repair deficiency in rodent cells by transfection with purified human DNA; (4) isolation of functional human DNA repair genes from cosmid and cDNA libraries; (5) construction of unique shuttle vectors for the analysis of gene expression and genetic recombination; and (6) model cultured cell systems (including primary human fibroblasts) for determination of low-dose rate radiation effects. This research

contributes to our understanding of the complex relationship between DNA damage and repair and the process of gene mutation and the initiation and progression of neoplasia. Future research will be focused on: (1) the nature of cellular mechanisms mediating the repair of radiation- and chemical-induced DNA damage; (2) the characterization of human repair genes and their encoded proteins; and (3) consequences of low-dose rate radiation exposure at the molecular and cellular levels.

Research Accomplishments:

· In collaborative effort we have determined mutation induction frequencies as a function of LET (10-2000 KeV/μm), confirming the notion that high LET radiation is more efficient for mutation induction.

· More than two hundred x-ray-, α-particles-, and heavy ion-induced human HGPRT deficient mutants have been isolated. Analysis of the type and extent of these mutations is in progress.

· Some chromosomal exchange-type aberrations, previously thought to occur through the passage of single charged particle tracks (e.g., isochromatid types), have been shown to be produced by a "two-hit" mechanism.

· We have observed that most radiation-induced chromosomal interchanges require damage to both chromosomes involved in the aberration, in contrast to models of aberration formation that are based on homologous recombination.

· We have observed that the kinetics of break rejoining in PCC (following exposure to γ-rays) does not support the view that the rate of chromosomal repair is dependent on dose.

· We have isolated and purified a siderophore polypeptide from a species of *Pseudomonas*.

· A *Pseudomonas*-derived siderophore polypeptide has been used in initial formation constant experiments using both Fe^{3+} and $^{239}Pu^{4+}$.

· Several preliminary experiments were conducted on the biodegradation of TCE by different species of microorganisms.

· We have acquired two CHO cell lines that differ in their nuclear morphology during growth, with one being fairly flat (mean thickness ~5 μm) and the other remaining nearly spherical (mean thickness ~10 μm). The RBE for 0.28 KeV x-rays is much higher for the thicker cell, even though these two cells are nearly identical in their response to gamma-irradiation. This result confirms our earlier finding of a dependance of RBE on nuclear thickness using different cell lines.

· Using a modified ferrous-ferric dosimetry system, we have made the first experimental measurement of the G-value for 1.5 KeV x-rays. In agreement with theory, this value was ~50% of the G-value for high energy gamma-rays. This confirmation is critical to current models of the mechanisms of radiation damage in biological systems.

· Using both V79 and CHO cells, we have found no statistically-significant variation in OER as a function of dose, over the range from 80% survival to 0.1% survival. We have also found that using the standard linear-quadratic survival model can lead to calculation of OER differences that are not objectively present in the actual data.

· We have designed and constructed a ^{238}Pu α-particle source for exposing monolayer cultures. This source incorporates a series of mylar attenuators that can alter the incident α-particle energy from ~4 MeV down to essentially zero. The source also has a camera shutter and timer, allowing accurate and reproducible exposures down to less than 1 cGy.

· The induction of genetic recombination in Chinese hamster ovary cells by ultraviolet light and ^{60}Co γ-rays has been detected with a shuttle vector system.

· A new procedure for the cloning of mammalian cell genomic DNA that enables restriction endonuclease digestion of DNA in cell lysates without further purification has been developed and used to construct genomic DNA cosmid libraries.

· New shuttle vector systems have been constructed to examine the effects of transcriptional activity on genetic recombination in mammalian cells.

· New procedures with increased sensitivity for the detection of pyrimidine dimers and closely opposed pyrimidine dimers in mammalian genomic DNA have been developed and characterized.

· Optimal conditions for the transformation of Chinese hamster ovary cells by electroporation have been determined.

· Transverse alternating field electrophoresis (TAFE) has been employed to measure significant differences in the migration of high molecular weight chromosomal DNA from irradiated DNA repair-proficient mammalian cells versus DNA repair-deficient cells 1 hr postirradiation.

· Cell survival determinations indicate a direct correlation between ionizing radiation sensitivity and dose rate in DNA repair-proficient mammalian cells, a feature which is lacking in DNA repair-deficient cells.

· A selection method, which eliminates the expression of revertant cell populations during DNA (cDNA)-mediated gene transfer, has been developed in the selection of ionizing radiation repair-proficient transformants.

· In collaborative studies, we have identified four new genetic complementation groups for the repair of ionizing radiation-induced DNA damage using rodent-human cell hybrid constructs.

· Using Southern blotting techniques, we have determined that gene mutations induced in excision repair-deficient mammalian cells at the X-linked HPRT locus by N-2-acetoxyacetylaminofluorene are either base change or small deletion events.

· The polymerase chain reaction (PCR) technique has been used to amplify cDNA from $HPRT^-$ mammalian cell mutants, which is allowing us the possibility of directly sequencing mutated genes and determination of location of base change on small deletion events.

· A number of genetic approaches showed that the putative ERCC5 human DNA repair gene was inherited within a unique secondary UV^r, Max^r cotransformant of UV-135 cells. Southern blot analysis indicated the presence of 100-200 kbp of human DNA and 5 copies of cotransferred pSV2gpt DNA within this cotransformant.

· We have succeeded in isolating a *functional* group of clones (3 out of a total of 85 containing human DNA) of the ERCC5 gene from an S-cos cosmid DNA library. The functional clones confer very high level cotransfer of UV^r and neo^r (a marker gene on the cosmid) into UV-135 cells.

· Southern blot analysis with a probe from active cosmids containing the putative ERCC5 gene showed that their human DNA was inherited with complete consistency with the UV resistance phenotype.

PULMONARY BIOLOGY

Project Descriptions:

Mechanisms of Pulmonary Damage (DOE)

Principal Investigator: B.E. Lehnert
Co-Investigators: J.A. Steinkamp, L.R. Gurley, R.A. Tobey
LS Collaborators: G.C. Saunders, H.A. Crissman, J.L. Cooper, D.M. Stavert, J.E. London, T.N. Buican, J.H. Jett

The objective of this project is to delineate the underlying biophysical, cellular, and molecular mechanisms involved in lung defense, lung injury, and lung repair. Work in the area of lung defense focuses on identifying and characterizing subpopulations of pulmonary macrophages in the context of: (1) cell-mediated particle clearance from the lung, (2) mechanisms by which pulmonary macrophages mediate injury to other lung cell types following the inhalation of environmental insults, (3) tumor surveillance and tumoricidal activities of pulmonary macrophages, and (4) macrophage regulation of fibroblasts during lung repair processes. The lung repair component of the project is concerned with the elucidation of cell-cell interactions and cytokines, or mediators, involved in modulating the recruitment, proliferation, and collagen synthesizing activities of lung fibroblasts during the development and progression of pulmonary fibrosis. The lung injury component of the project focuses on lung free cell alterations caused by environmental insults to the lung and on permeability changes in the lung's air-blood barrier following such insults. The experimental approaches used in this project utilize both *in vivo* and *in vitro* model systems, and involve techniques that include multiparameter flow cytometry, high performance liquid chromatography, and electron microscopy.

Evaluation of NO_x-Induced Toxicity (MRDC)

Principal Investigator: B. E. Lehnert
Co-Investigator: D. M. Stavert
LS Collaborators: L. R. Gurley, J. E. London

This project is concerned with identifying risk factors that are involved in producing respiratory tract and extra-pulmonary disorders upon exposure to high mass concentrations of nitric oxide (NO) and nitrogen dioxide (NO_2), both alone and in combination. Current activities of this ongoing project include: (1) continued assessments of NO_2 concentration-exposure time relationships relative to the kinetics and magnitude of respiratory tract injury, (2) evaluations of the severity of NO_x-induced pulmonary and extra-pulmonary abnormalities as a function of minute ventilation, (3) assessments of exercise as a potentiator of NO_2-induced lung injury using NO_2 concentrations that are greater and lesser than those previously studied in our laboratory, (4) the delineation of relationships between exercise intensity and the magnitude of potentiation of lung injury, (5) investigations of the mechanism by which exercise potentiates NO_2-induced lung injury, (6) further investigations of relationship(s) among maximum oxygen consumption (VO_{2max}) during exercise, blood lactate accumulation rates, and exercise times and intensities to determine if VO_{2max} values correlate with sub-strenuous exercise-associated fatigue, (7) continuation of investigations on the role methemoglobin formation has on work performance degradation following the inhalation of high concentrations of NO, and (8)

assessments of alterations in ventilatory function during exposure to high concentrations of NO_2 for the purpose of dosimetry modeling and extrapolation to the human condition. The results of these studies will substantially contribute to the understanding of processes involved in NO_x-induced injury, as well as lung injury caused by the inhalation of oxidant gases in general, and thereby provide rational bases for the future development of prophylactic and therapeutic strategies for ameliorating oxidant gas-induced injury.

Acute Inhalation Toxicity of Pyrolysis Products of Halon 1301 (MRDC)

Principal Investigator: B. E. Lehnert
Co-Investigator: D. M. Stavert
LS Collaborator: J. E. London

This project investigates the pathophysiology due to the acute inhalation of hydrogen fluoride (HF), hydrogen bromide (HBr), and hydrogen chloride (HCl) vapors that can be generated from the thermal decomposition of the fire retardant Halon 1301. The specific objectives of the project are to (1) characterize the toxicologic effects induced in the respiratory tract by acute exposures to HF, HBr, and HCl, alone and in combination; (2) determine how exposures to these agents and mixtures of these substances alter normal breathing patterns and pulmonary functional status; (3) evaluate how altered minute ventilation during exposure to these agents may impact on the expression of respiratory tract injury; (4) investigate relationships between acute exposures to the halides and work performance capacity thereafter; and (5) determine how work performance, or exercise, following exposures to HF, HBr, and HCl may modify the severity of respiratory tract injury. These studies will provide an extensive data base on the toxic responses to the above irritant gases while providing fundamental information on the pathophysiologic processes that underlie gas-induced respiratory tract injuries.

Research Accomplishments:

· A high T_c superconductor material composed of yttrium-barium-copper oxide has been found to produce pulmonary fibrosis and a bronchoepithelialization of the alveoli of the rat's lung. Our findings suggest that yttrium-barium-copper oxide may represent a previously unidentified hazard to the lung.

· Prostaglandin E_2 can inhibit lung fibroblast proliferation *in vitro*. Using human lung fibroblasts, we have found that PGE_2 causes only a transitory delay in fibroblast proliferation. Our results suggest that PGE_2 that is elaborated by alveolar macrophages during pulmonary fibrogenesis may not be involved in the regulation of lung fibroblast proliferation *in vivo*.

· The migratory behavior of alveolar macrophages (AM) were assessed as a function of cellular burdens of particles they contained. The results suggest that AM migration in the lung may be substantially reduced following the deposition of high lung burdens of particles.

· Pulmonary fibrosis and cancer can occur following the lung deposition of high burdens of even relatively innocuous particles. We have found that polymorphonuclear leukocytes (PMN) in the lung are evaluated under conditions where lung clearance is compromised by excessive lung burdens of non-cytotoxic particles. Our results suggest a role for PMN in causing pathologic changes observed in particle overload conditions.

· The retention of particles that deposit in the lung often can be described as a multi-component process. We have found that the disappearance of particle-containing alveolar macrophages (AM) over the course of lung clearance of a low lung burden of insoluble particles is also multiphasic.

· Airway intra-luminal macrophages have been viably isolated from cells lavaged from the conducting airways of the rat using multiparameter flow cytometry.

· Particle distributions in airway intra-luminal macrophages were compared to those in alveolar macrophages during the clearance of polystyrene microspheres from the lung. Particle distributions in the airway macrophages were found to be virtually identical to those in the alveolar macrophages. These findings indicate airway macrophages represent alveolar macrophages that are undergoing translocation from the lung.

· An IgM monoclonal antibody that binds to the surface of lung fibroblasts is being used in conjunction with multiparameter flow cytometry to isolate viable lung fibroblasts from single cell suspensions of rat lung.

· We have found that respiratory tract injury following the inhalation of high mass concentrations of HF, HBr, and HCl by the obligatory nose breathing rat is confined exclusively to the nasal compartment. In order of relative toxicities, HF has been found to be more toxic than HBr or HCl.

· The use of the laboratory rat for inhalation toxicology studies is limited in that the rat is an obligatory nose breather. We have developed a rat model, which is called the "pseudo-mouth" breathing rat model, in which rats can be exposed to atmospheres of interest via the oral route. The pulmonary functional status of these rats is essentially identical to that of rats breathing normally through the nose.

· A toxico-kinetic model has been developed that predicts both the rate of accumulation of methemoglobin formation during the exposure of rats to nitric oxide (NO) and the rate of methemoglobin elimination following cessation of NO exposure.

· Work performance capacity has been demonstrated to be inversely proportional to the percentage of methemoglobin formed in the blood due to the inhalation of nitric oxide.

· Our HPLC approach for resolving and quantitating protein constituents in lung lavage fluids has been used to detect and characterize the status of nitrogen dioxide-induced permeability pulmonary edema in the rat's lung. Additionally, a major protein constituent in lung lavage fluids that is resolved by the HPLC technique has been identified to be transferrin.

· Electron microscopic and morphometric analyses have revealed that pulmonary interstitial macrophages isolated from single cell suspensions of rat lungs are more akin to blood monocytes than they are to lavaged alveolar macrophages.

· A system using partial body flow plethysmographs has been developed to measure the ventilatory functions of numerous rats concurrently during and after inhalation exposures to atmospheres of interest.

CARCINOGENESIS

Project Descriptions:

High Speed DNA Repair Genotyping (LANL)

Principal Investigator: M. A. MacInnes
Co-Investigator: R. T. Okinaka

The objective of this project is to develop rapid molecular tests to evaluate human genetic variability in DNA repair and associated mutation controlling mechanisms. Our approaches will ultimately make possible the elucidation of human genes and DNA sequences contributing to susceptibility to environmental genotoxins This study will evaluate the significance of certain regulatory regions of DNA repair genes as potential targets of mutations, and secondly, to evaluate methodologies of the polymerase chain reaction (PCR) technique for rapidly determining mutations in a known target DNA. Our analysis of gene regulatory mutations will help answer the question whether mammalian genes are more or less susceptible to this class of mutations in comparison to coding sequence mutations. The PCR technique will have broad applicability to problems of rapid sequence isolation and determination, without cloning, from genomic DNAs or mRNAs.

In Vivo Cytotoxic Activities of Immune Cells in Malignant Tumors (LANL)

Principal Investigator: R. A. Sneed
Co-Investigators: A. P. Stevenson, H. A. Crissman, C. C. Stewart

Our objectives are to identify the leukocytes that infiltrate selected malignant murine neoplasms and to define their infiltration kinetics and functions using multiparameter flow cytometry and fluorochrome-conjugated monoclonal antibodies. Two murine adenocarcinomas, one of high immunogenicity (EMT6) and a second of very low immunogenicity (Colon 26) were used. Tumors were grown in BALB/cJ mice and the kinetics of the host cell infiltrates were defined over a 28-day period. Among the findings was the marked difference in the quantity of the host cell infiltrates. In the EMT6 tumor, the number of host cells made up over 50% of all cells in a typical tumor. The number of host cells comprised an average of only 22% of total cells in the Colon 26 tumor. We are now developing *in vivo* techniques to monitor the effectiveness of adoptive immunotherapy in these two tumor types.

Karyotype Instability and Neoplasia (DOE)

Principal Investigator: P. M. Kraemer

We are investigating the role of immortalization and karyotype instability in the multistep neoplastic process. Using cell culture models of the neoplastic process, our specific aims include identification, detection, and characterization of genes and gene products that directly influence karyotype stability or cellular senescence and immortalization. Once these are identified, their role in the activation of protooncogenes, the suppression of recessive oncogenes, and other changes known to be involved in specific steps of neoplastic progression will be studied. We hypothesize that the driving force in the neoplastic process will turn out to be an ongoing genetic variability caused by alterations in these unidentified genes.

Utilization of Archival Tissues to Describe Alpha-Irradiation-Induced Lung Carcinogenesis (LANL)

Principal Investigator: J. S. Wilson

Two aspects of carcinogenesis are being studied: (1) specific, tumorigenic agent-induced changes associated with *in situ* tumors, and (2) changes occurring in fields of non-tumor cells in tumor-bearing tissues. Both flow cytometric analyses and DNA molecular assays are being used to determine the presence of DNA alterations associated with neoplastic progression. Recently developed methods to retrieve cells and nuclei from formalin-fixed, paraffin-embedded tissues provide opportunities to expand investigations of neoplastic progression. The use of archival tissue from extensive *in vivo* toxicological and tumorigenesis studies provides an immediate source of research material as well as eliminates the need for further animal exposures.

Applied Toxicology (DOE)

Principal Investigator: J. London

Using classical toxicology methods, various materials of interest to the Department of Energy are tested for toxic effects. The goal is to provide biological guidelines for establishing safe work environments. The objective is to provide standard operating procedures for working with particular materials. Parameters measured are (1) acute oral toxicity in rats and mice, (2) primary skin irritation in rabbits, (3) skin sensitization in rabbits, and (4) ocular inflammation in rabbits.

Research Accomplishments:

· DNA sequence analysis of the DNA repair gene ERCC1 showed that there are several sequence motifs not previously identified that may determine the gene's capacity to express in most or all tissues. Some of these potentially important sites will be mutagenized *in vitro* using synthetic oligonucleotide site-directed methodology and tested for phenotypic effect with a transient reporter gene expression assay

· We conducted initial experiments with the new polymerase chain reaction technique to rapidly identify mutations in a CHO chromosomal target gene (HGPRT). The specific method (cDNA PCR) detects the majority of point mutations which do not disrupt the expression and stability of the HGPRT messenger RNA. (A simple modification of the technique can be used to detect 5' regulatory sequence mutations.)

· Analysis of restriction enzyme site clusters identified the promoter region of the human repair gene XRCC1 which encodes a function involved in ionizing radiation resistance of cells *in vitro*.

· When we compared the immune responses of an immunogenic (EMT6) and a nonimmunogenic (Colon 26) murine tumor, we were able to show the presence of a concomitant immune response in the EMT6 tumor, but none in the Colon 26 tumor, even though its lymphocytes were proliferating.

· We found the approximately 50% of *in vivo* origin Colon 26 cells express the Thy 1.2 surface antigen (a murine T lymphocyte surface antigen) whereas *no vitro* cultured Colon 26 cells expressed this antigen. No EMT6 cells of either *in vivo* or *in vitro* origin expressed the Thy 1.2 surface antigen.

· We have used DNA fingerprinting techniques to monitor the loss of chromosomal stability during neoplasia. Early changes detected during the spontaneous transformation of cultured Chinese hamster cells include the loss of parental alleles and the detection of an extremely unstable locus.

· We have developed a retrievable sponge method for assaying tumorigenesis in nude mice. This method has proven to be important in studies of newly transformed human cells.

· We have made a plasmid construct containing the SV_{40} large T antigen gene, but lacking any other SV_{40} sequences. Transfection of this plasmid into human cells has shown that the primary role of this antigen in neoplastic transformation is to destabilize the genome. Neoplastic properties are acquired later in stepwise fashion and the cells ultimately become fully neoplastic.

Sponsors (Abbreviations)

LANL	Los Alamos National Laboratory
DOE	Department of Energy
OHER	Office of Health and Environmental Research
NIH	National Institutes of Health
NCI	National Cancer Institute
DoD	Department of Defense
MRDC	U.S. Army Medical Research and Development Command
ACRDEC	U.S. Army Chemical Research, Development, and Engineering Center
ARI	Army Research Institute
DOI	Department of the Interior
NASA	National Aeronautics and Space Administration
BES	Basic Energy Research
USDA	United States Department of Agriculture
ACS	American Cancer Society
NFCR	National Flow Cytometry Resource

FACILITIES

National Flow Cytometry Resource

L.S. Cram, M.F. Bartholdi, M. Cassidy, G.C. Salzman, C.C. Stewart

The National Flow Cytometry Resource (NFCR) was established to make state-of-the-art flow cytometric instrumentation available to the biomedical research community. The NFCR is supported by the Biomedical Research Technology Program, Division of Research Resources of NIH and by the Office of Health and Environmental Research, Department of Energy. The focus of the NFCR is to provide an active and dynamic program of collaborative research, develop new instrumentation capabilities and biological techniques based on identified user need, train scientists in the use of flow cytometry, and serve as an interface between the academic, private and government sectors. To this end the Resource has five major goals:

- **Technological Research and Development**. Develop and make available advanced flow cytometric instrumentation and relevant methodology in response to specific needs of the biomedical community, collaborators, and service users. Identify new investigative and clinical uses for flow cytometry.
- **Collaborative Research**. Establish research projects between Resource staff and scientists at other institutions that enhance and enlarge the frontiers of flow cytometry for answering significant biomedical questions.
- **Service Projects**. Provide support for short-term projects to the "user" community that will help facilitate the availability of general flow cytometric techniques and technology.
- **Training**. Students, postdoctoral appointees and scientists are trained in the operation and application of flow instruments and in related technologies.
- **Dissemination.** Information about the availability of the NFCR and its activities is disseminated through the Flow Systems Newsletter, postdoctoral training, brochures, and transfer of technology to other institutions.

Unique capabilities either exist or are being established in the following areas: chromosome image analysis and sorting, high resolution analysis, super high speed sorting, and multilaser excitation systems. Techniques include: analysis of fluorescence polarization, kinetic measurements, correlated data analysis procedures, high resolution DNA studies, staining protocol development, fluorescence distribution analysis, and multiparameter procedures. Research fields include immunology, cell biology, gerontology, tumor biology, infectious disease, parasitology, molecular biology, and radiobiology. Biomedical research investigators from around the nation are involved in the use and management of this user designated facility.

The Resource facilities are centered around five instruments:

1. Multiparameter Flow Cytometer: Combines computer-based eight-parameter data acquisition, Coulter dc volume analysis, five-color fluorescence, small-angle light scatter, axial light loss, autofluorescence correction, cell sorting, and time and temperature as parameters for kinetic measurements.
2. Chromosome High Resolution Imaging Sorter: Provides imaging in flow for detecting aberrant chromosome types, dual channel pulse shape recording and high resolution DNA analysis. Chromosome sorting based on morphological descriptors is in place and sorting based on *in-situ* hybridization probes is under development.

3. High Speed Sorter: Provides high speed chromosome sorting, rare event analysis, dual-laser excitation, and precise laser monitored single droplet charging.

4. EPICS V: This instrument is a dual laser-based flow cytometer/ sorter used primarily for chromosome analysis and sorting for the NFCR, the National Laboratory Gene Library Project (funded by DOE) and the Dot-Blot Resource (funded by NIH).

5. Becton-Dickinson FACS II: Provides dual-laser excitation, dual-color fluorescence measurement, small-angle light scatter, and large particle and four-way sorting.

The Resource contains unusual computing capabilities; each of the flow instruments has a dedicated computer for data acquisition and analysis. Data processing and computer graphics are available through the Division's Flo-VAX system and through a direct tie line to the Laboratory's Central Computing Facility.

In addition, to the above, two unique capabilities are under development: Fourier transform spectral analysis and phase sensitive detection. Both capabilities are designed to extend the information gathering capabilities of multiparameter flow cytometers. The Fourier transform flow cytometer is now operable and has been used to measure a shift in Nile red fluorescence accompanying the induced differentiation of 3T3 fibroblasts to adipocytes. A phase sensitive flow cytometer is under construction for measuring the fluorescence decay time of multiple fluorochromes with similar emission spectra, thereby increasing our ability to resolve multiple fluorescent tags on a single cell.

Other development highlights during the year include the use of sorted chromosomes and bivariate flow karyotype analysis in cancer research, the analysis of leukocyte infiltration into solid tumors, the development of new clustering algorithms for analyzing six and eight parameter data, and the development and implementation of a color enhanced data analysis package: "Brush Plot." Most of these developments are described elsewhere in the annual report.

National Laboratory Gene Library Project

L. L. Deaven, C. E. Hildebrand, and L. S. Cram

The National Laboratory Gene Library project was initiated at the Los Alamos and Lawrence Livermore National Laboratories in 1983. The aim of this resource is to provide interested scientists with chromosome-enriched DNA libraries that cover the entire human genome. Both laboratories have excellent flow-sorting instrumentation and years of experience in the use of sorted chromosomes for biological studies, and both laboratories have received numerous requests over the past few years to provide purified chromosomes to investigators for use in their own research. Accordingly, we felt that the availability of chromosome-enriched libraries would have a major impact in studies of the human genome and that it would be more efficient to construct these libraries for general distribution rather than to purify chromosomes for each investigator. Personnel at the U. S. Department of Energy, our prime contractor, agreed with this assessment and initiated this project as a collaborative effort between the two laboratories.

In order to develop goals and strategies for this project, we sought input from the scientific community at both national and international meetings as to the types of libraries to be constructed and in setting priorities. In addition, an advisory board continues to provide us guidance. This board consists of Paul Berg (Stanford), Tom Maniatis and Sam Latt (Harvard), Arno Motulsky (University of Washington), Bill

Rutter, (University of California, San Francisco), Carl Schmid (University of California, Davis), Tom Shows (Roswell Park), Tom Caskey (Baylor), Richard Gelinas (University of Washington), and Fred Blattner (University of Wisconsin). Integration of suggestions from our advisory board and from the scientific community resulted in the division of this project into phases. Phase I is aimed primarily towards the needs of the medical genetics community and involves production of complete digest libraries. Phase II supports research of the molecular biology community by the production of libraries with large inserts in one of the newer lambda and cosmid vectors.

The Phase I libraries are now completed and are available to user groups through an NIH sponsored repository at the American Type Culture Collection, Rockville, MD. The first partial digest libraries for chromosomes X, 16, 19, 21, and 22 are also available from ATCC. Currently, we are completing the construction of one cosmid and one phage library for each of the remaining human chromosomes.

We believe the libraries will have a positive impact in the following areas of human genetics research:

- Gene mapping;
- Linkage and pedigree analysis using DNA restriction fragment-length polymorphisms;
- Study of chromosome-specific alteratiSammons associated with inborn genetic and neoplastic disorders;
- Comparative study of DNA sequence organization among individual chromosomes within a given species;
- Interspecies genetic comparisons, such as the study of linkage group homologies and rearrangements during evolution; and
- Basic molecular genetic studies of gene structure, arrangement, and expression.

MicroVAX Computer System

J. H. Jett and M. E. Wilder

A MicroVAX II based computer system has been purchased with the aid of an NIH Shared Instrumentation Grant and consists now of 6 computers operating in a cluster mode. The backbone of the system is the ETHERNET communications protocol that ties the computers and the users together. All computers, and users of the computers, communicate with the cluster over the ETHERNET. In the cluster mode, one MicroVAX II, with the two RA-81 disks connected to it, serves as the cluster boot mode. This allows all software used to reside in one place for ease of maintenance. In addition, all user files reside on an RA-81 disk. At the present time, the user disk is 98% full. A second user disk will be added in the near future. This configuration has proven to be very user-friendly and easy to control.

This system has proven to be extremely reliable. It is available to users 24 hours a day, 7 days a week. With only one or two exceptions, the only time that the system was not available during the past year was when the monthly complete system back-up was being made. The cluster uses the VMS operating system in the cluster mode with the DEC Local Area Transport protocol to provide users throughout the Life Sciences building with communications services to the system. The DECNET protocol is used to communicate with the Laboratory's Central Computer Facility (CCF). A special communication program has been written under contract to provide a file transfer capability between the data collection computers at the flow cytometers and the MicroVAX cluster. This program (RT2VAX) is capable of transferring files over the ETHERNET at speeds up to 120 kbaud. Several commercial graphics packages have been installed including DISSPLA, SAS/GRAPH, PLOT 10 and the Digital Equipment Workstation software. The connection to the Laboratory Central Computing Facility has been

completed and is operational. This allows users to use the Cray-level computing power available in the CCF as well as the high resolution graphics capabilities. The Laboratory high- level graphics program MAPPER is being used extensively for development of data display formats.

Several programs for flow cytometric data analysis and display have been either converted or written for VAX system operation. The list mode processing programs that produce univariate and bivariate histogram displays are available. They are the most highly used data analysis programs. The several cell cycle distribution analysis programs have been integrated into one program for the VAX system. A new clustering program has been written that uses the color graphics work station that is part of the system. The program displays the clustering results as multiple bivariate dot-plots that are color coded by cluster number. The program will display selectively as many clusters as the operator desires, allowing one to fully investigate the results of the clustering using the color graphics capabilities.

Electron Microscopy Facility

R. J. Sebring

Among the sophisticated resources within Life Sciences Division is the electron microscopy (EM) facility which provides electron microscopic support to the Pulmonary Program and to the Life Sciences Division generally. This facility has both scanning (SEM) and transmission (TEM) microscopes, the latter being equipped with energy dispersive spectroscopy (EDS) for elemental analysis of thin sections. An image analysis system extends the quantitative capabilities of the facility to images produced by the SEM, TEM, and light microscopes. In addition to imaging and analysis capabilities, this facility has the following: a modern, fully equipped, preparative laboratory for performing biological specimen preparations, and a functional photographic darkroom for processing EM negatives and producing electron micrographs for presentation and publication.

Nuclear Magnetic Resonance Facility

L. O. Sillerud

The Biomedical Nuclear Magnetic Resonance (NMR) Facility is located in HRL 244/246 and consists of a Bruker AM400wb superconducting multinuclear spectrometer. We have the capability of observing nuclei that resonate from silver-109 to phosphorus-31, and protons. We can accommodate samples ranging in size from 5 mm (protons) to 20 mm (^{109}Ag-^{31}P) in standard sample tubes. We have recently installed proton imaging for samples from 3 to 25 mm diameter. The imaging resolution can be as good as 8 μm. We are currently developing heteronuclear, inverse imaging methods for the imaging of the metabolism of added nuclei, such as ^{13}C.

High- and Low-Dose Rate Gamma-Ray Irradiation Facility

G. F. Strniste

Recently, a dual irradiation facility was completed in the Life Sciences Division containing a high-dose rate ^{60}Co and a low-dose rate ^{137}Cs gamma-irradiator. Capital equipment funds for the procurement of the two irradiators came from a LANL Institutional Support and Research Development award and the LS Division. Construction funds were obtained from the previous Chemistry, Earth and Life Sciences

Associate Directorate and the Life Sciences Division which provided the necessary finances to renovate and upgrade a radiation source room in the HRL-1 Building in order to accommodate the two gamma-ray sources. The high-dose rate irradiator is a reconditioned, Picker Cyclops Radiotherapy unit containing approximately 5000 Ci of ^{60}Co (as of 11/86). This unit generates an intense beam of 1.17-MeV gamma rays at a dose rate of ~77 cGy (rads) per minute at a distance of one meter from the source (determined during 10/87). The low-dose rate irradiator is a specially designed Model 81-12 unit (purchased from J. L. Shepherd and Associates, San Fernando, CA) containing both a 10 Ci and a 1000 Ci ^{137}Cs source. A beam of 0.66-MeV gamma rays at dose rates between 0.2 and 200 cGy per hour at one meter from the source (measured during 02/87) is achievable with this dual source unit. Both irradiators are used by radiobiologists in the Life Sciences Division in experiments designed to explore mechanisms of radiation action at the molecular, cellular, and organismal levels.

Cell Farm

J. G. Tesmer

The cell farm was started in 1960 to provide a central media and CHO cell supply. Today, the cell farm has grown from an organization supplying one type of medium and one cell line to a large Division-wide service facility. We supply sterile glassware, filter almost 7000 liters of media a year consisting of 30 different cell culture-related solutions, and provide a wide variety of cell lines in spinner and monolayers as requested. The cell farm also serves as a resource for cell culture information and handles serum testing and purchases.

Four technicians staff the cell farm in the LS-3 Group. Some of the technicians provide technical support to projects in LS-3, concerned with mammalian cell organization and expression, and to the Radiation Genetics Section.

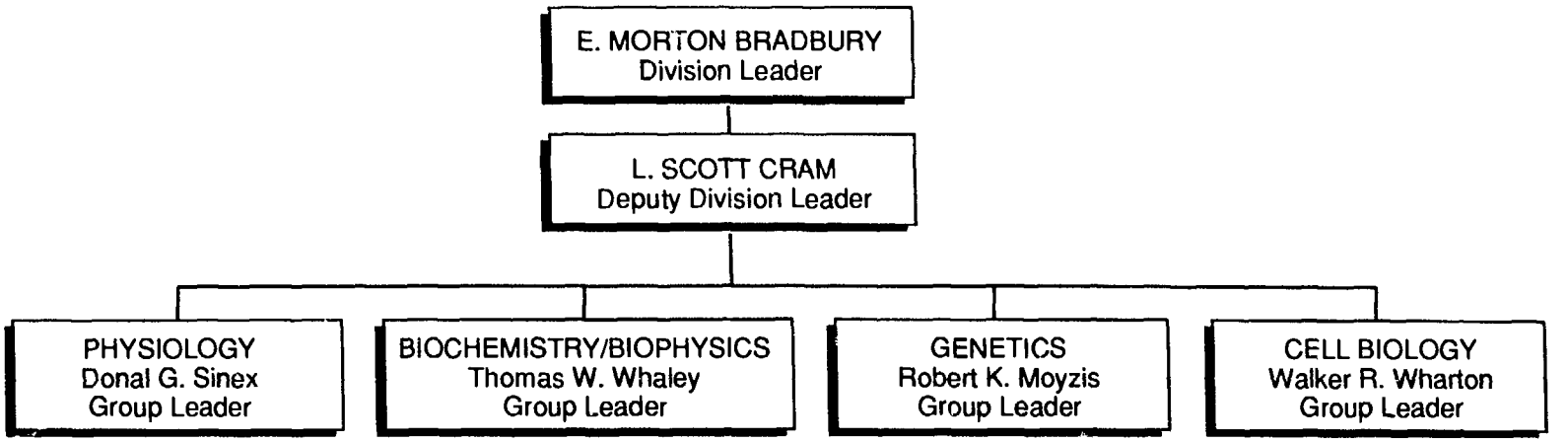
Animal Care Facility

T.W. Whaley

The Animal Care Facility is a support organization for the Life Sciences Division's research programs. It is accredited by the American Association for Accreditation of Laboratory Animal Care. The institution's ten-member Animal Care and Use Committee reports to the Director of the Laboratory and provides independent oversight and review of the Division's research involving animals. The Animal Care Facility is staffed with an attending veterinarian, a facility manager, and 7 animal caretaker personnel. During the past year, approximately 12,000 animals were housed in the facility. The vast majority of the animals were rats and mice, but a variety of other species were also housed in the facility. In the past year, these other animals included monkeys, hamsters, guinea pigs, chinchillas, ferrets, chickens, rabbits, sheep, frogs, and toads. A nude mouse breeding colony, which produces approximately 1000 animals per year, has been maintained continuously in the Division for 10 years. The physical plant of the facility consists of approximately 8000 square feet of space in the Health Research Laboratory main building and an additional 4000 square feet of space at four outlying sites at the Laboratory. Renovation of the main HRL facility is planned in the upcoming year.

ORGANIZATION

LS DIVISION ORGANIZATIONAL CHART



NEUROBIOLOGY SECTION
- Neuropharmacology
- Microwave Effects
- Phototransduction
- Magnetoencephalography
- Auditory Neurophysiology

PULMONARY SECTION
- Pulmonary Biology-Toxicology
- Electron Microscopy
- Biocompatible/ Weapons Tox.

STRUCTURAL BIOLOGY SECTION
- Neutron Scattering
- X-Ray Diffraction

BIOCHEMISTRY SECTION
- Nucleoprotein Chemistry
- Diabetes Studies
- Microbial Biochemistry
- Magnetic Resonance Spect.

BIOPHYSICS SECTION
- Light Scattering
- Instrument Development
- Artificial Intelligence

ANIMAL COLONY SECTION

GENOME ORGANIZATION SECTION
- Mapping Human Genome
- Gene Organization
- DNA Replic. & Amplification
- Molecular Cytogenetics

GENE EXPRESSIONS SECTION
- DNA-Protein Interactions
- Plant Genetics
- Salt Tolerance

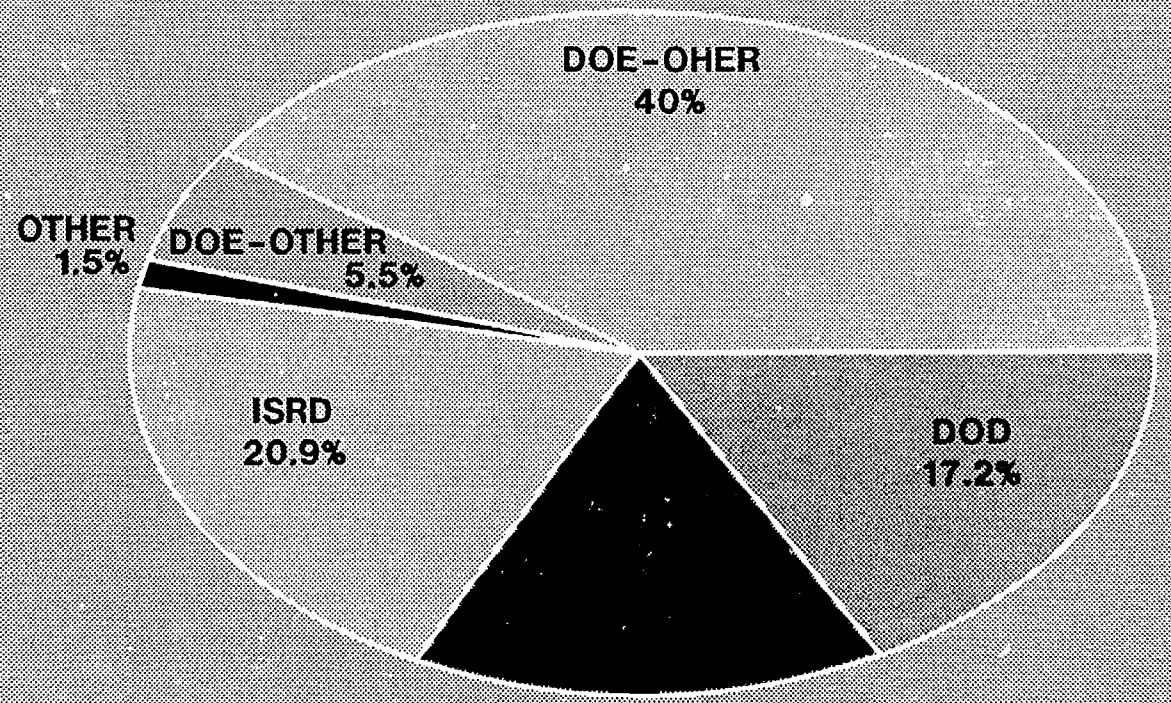
RADIATION GENETICS SECTION
- DNA Damage
- DNA Repair Genes

CELL FARM SECTION

- Cell Cycle
- Tumorigenesis
- Immunology
- Flow Cytometry
- Radiobiology
- Reproductive Cell Biology
- Cytogenetics
- Knowledge Based Systems
- Ultrasensitive Detection
- Chromosome Library

72-114

FUNDING DISTRIBUTION
LIFE SCIENCES DIVISION
FY89



Los Alamos

LIFE SCIENCES DIVISION (LS-DO)

E.M. Bradbury Division Leader
L. S. Cram, Deputy Division Leader
C. G. Stafford, Assistant Division Leader/Finances

D. G. Sinex, Group Leader, LS-1
T. W. Whaley, Group Leader, LS-2
R. K. Moyzis, Group Leader, LS-3
W. R. Wharton, Group Leader, LS-4
D. L. Spitzmiller, Administrative Assistant
M. C. Cassidy, Technical Coordinator/Personnel Specialist
J. L. Horne, Property/Engineering Manager
D. Quintana, Student Administrative Aide
W. F. Marek, Student Property Aide
W. Scoggins, High School Co-op

PHYSIOLOGY GROUP (LS-1)

D. G. Sinex, Group Leader
J. Lujan and N. Scott, Executive Secretaries

Staff Members

C. J. Aine
J. C. Fowler
J. S. George
D. L. Hjeresen
B. E. Lehnert
J. E. London
J. M. O'Donnell
D. M. Stavert
M. M. Whalen
A. Yamazaki

Technical Staff

D. C. Archuleta
A. F. Cline
E. A. Margiotta
M. Martinez
L. P. McDonald
R. J. Sebring
J. J. Stimmel
K. O. Umbarger
Y. E. Valdez

Post-Doctoral Appointees

J. F. McElroy
J. B. Mott

Students and Assistants

D. T. Garcia
R. E. Mischke
J. B. Ortiz
A. L. Petteway
K. E. Toevs

Distinguished Visitors

F. Hayashi
Department of Biology
Kobe University
Nada, Kobe
Japan

D. Shockley
Meharry Medical College
Nashville, TN

BIOCHEMISTRY/BIOPHYSICS GROUP (LS-2)

T. W. Whaley, Group Leader
G. K. Coffin, Executive Secretary

Staff Members

M. R. Brooks
D. L. Grady
L. R. Gurley
C.-H. Han
L. W. Hersman
R. G. Johnston
A. Kowluru
R. Kowluru
G. C. Salzman
A. G. Saponara
L. O. Sillerud
J. Trewhella

Technical Staff

N.H. Fink
W.K Grace
N. Lehnert
S.E. Rokop
J.A. Salazar
J.G. Valdez

Post-Doctoral Appointees

D. B. Heidorn
W. K. Liddle
T. Sosnick

Students and Assistants

S. Bhatia
M. Chan
K. L. Coulter
J. Hammer
R. Lehndorf
C. Lemanski
C. Kirsch
L. Swanson
D. Vigil

Animal Care Section

P. Aguilar
M. J. Behr
M. E. Camp
J. G. Graeber
L. R. Gurule
A. J. Herrera
J. A. Jaramillo
G. A. Maestas

GENETICS GROUP (LS-3)

R. K. Moyzis, Group Leader
C. E. Hildebrand, Deputy Group Leader
G. F. Strniste, Technical Coordinator
M. H. Fink, Administrative Assistant
P. R. Schmeiser, Senior Secretary

Staff Members

D. J-C. Chen
S. H. Cox
J. A. D'Anna
J. W. Heyser
P. J. Jackson
J. L. Longmire
M. A. MacInnes
J. Meyne
R. T. Okinaka
R. L. Ratliff
R. J. Reynolds
R. L. Stallings
R. A. Tobey
J. S. Wilson

Technical Staff

N. C. Brown
J. M. Buckingham
V. L. Church
L. M. Clark
A. A. Ford
M. D. Jones
T. T. Lucero
J. A. Luke
L. J. Meincke
A. C. Munk
J. W. Nickols
D. L. Robinson
E. P. Saunders
J. G. Tesmer
E. C. Wilmoth

Post-Doctoral Appointees

E. Delhaize
J. Dietz-Band
L. S. McCoy
J. Mudgett
J. Nickoloff
M. Yu

Students and Assistants

S. L. Anzick
J. M. Berger
M. J. Chacon
F. T. Chavez
D. Corvo
D. de Bruin
S. L. Fehrenbach
C. L. Hardekopf
S. R. Hardison
C. Heighway
P. Honsinger
M. Huang
P. Y. Hsue
S. D. Jett
A. K. Lewis
L. Lujan
P. N. Martinez
M. M. Rodriguez
L. Spirio

Distinguished Visitors

P. Jeggo
National Institute for
Medical Research
London, England

R. Johnson
Howard University
Washington, D.C.

H. Nagasawa
Harvard School of
Public Health
Boston, MA

J. R. Wu
Johns Hopkins University
Baltimore, MD

M. Wu
University of Maryland
Baltimore, MD

CELL BIOLOGY GROUP (LS-4)

W. R. Wharton, Group Leader
J. H. Jett, Deputy Group Leader
R. R. Archuleta, Executive Secretary
M. C. Cassidy, National Flow Cytometry Resource

Staff Members

M. F. Bartholdi
T. N. Buican
E. W. Campbell
S. G. Carpenter
J. L. Cooper
M. N. Cornforth
H. A. Crissman
L. L. Deaven
J. P. Freyer
R. D. Hiebert
P. M. Kraemer
B. L. Marrone
J. C. Martin
M. R. Raju
F. A. Ray
G. C. Saunders
J. A. Steinkamp
A. P. Stevenson
C. C. Stewart
M. E. Wilder

Technical Staff

K. L. Albright
S. Bailey
C. Bell-Prince
B. F. Bentley
J. J. Fawcett
R. C. Habbersett
M. L. Leudemann
A. Martinez
J. D. Parson
S. J. Stewart
P. L. Schor
K. J. Thompson

Post-Doctoral Appointees

L. A. Krakowski
M. Smyth
R. Sneed

Students and Assistants

K. Dahlby
J. Folz
B. Hoffman
C. Hudson
L. Hutson
D. Kim
J. Lopez
K. McGee
M. Naiver
B. Stone
M. Tinkham
M. Trent
R. Wynn

Distinguished Visitors

N. Oishi
University of Hawaii
Honolulu, Hawaii

COMMUNICATIONS

PUBLICATIONS

Akporiaye, E. T., M. Kudalore, A. P. Stevenson, P. M. Kraemer, and C. C. Stewart. Isolation and reactivity of host effectors associated with the manifestation of concomitant tumor immunity, *Cancer Research* **48**, 1153-1158 (1988).

Antao, V. P., D. M. Gray, and R. L. Ratliff. CD of six different conformational rearrangements of poly d(A-G)·d(C-T) induced by low pH, *Nucleic Acids Res.* **16**, 719-738 (1988).

Bartholdi, M. F., J. Meyne, R. G. Johnston, and L. S. Cram. Chromosome banding analysis by slit scan flow cytometry, *Cytometry*, in press.

Berger, J. M., P. J. Jackson, N. J. Robinson, L. D. Lujan, E. Delhaize. Precursor-product relationships of poly(γ-glutamylcysteinyl)glycine biosynthesis in *Datura innoxia, Plant Cell Rep.*, in press.

Blinder, M. A., J. C. Marasa, C. H. Reynold, L. L. Deaven, and D. M. Tollefsen. Heparin cofactor II: cDNA sequence, chromosome localization, restriction fragment length polymorphism, and expression in *Escherichia coli, Biochemistry* **27**, 752-758 (1988).

Bohren, C. F. Scattering by a sphere and reflection by a slab: some notable similarities (Letters to the Editor), *Applied Optics* **27** (2), 205-206.

Cook, G. R., M. Minch, G. P. Schroth, and E. M. Bradbury. Analysis of the binding of high mobility group protein 17 to the nucleosome core particle by ^{1}H-NMR spectroscopy, *Journal of Biological Chemistry*, in press.

Cooney, C. A., R. L. Eykholt, and E. M. Bradbury. Methylation is coordinated on the putative replication origins of *Physarum* rDNA, *Journal of Molecular Biology* **204**, in press.

Cooper, J. L., E. Wakshull, and W. Wharton. Characterization of the rebinding of ^{125}I-epidermal growth factor released from BALB/c-3T3 cells following accumulation in the presence of chloroquine, *J. of Cell. Physiol.* **134**, 387-395 (1988).

Crabb, D. W., L. L. Deaven, M. L. Luedemann, B. Zhang, and R. A. Harris. Assignment of the gene for the E1α subunit of branched chain α-ketoacid dehydrogenase to chromosome 19, *Cytogenetics and Cell Genetics*, in press.

Crissman, H. A., M. H. Hofland, A. P. Stevenson, M. E. Wilder, and R. A. Tobey. Use of DiO-C_5-3 to improve Hoechst uptake, resolution of DNA content and survival of CHO cells, *Exp. Cell Res.* **174**, 388-396 (1988).

Crissman, H. A., M. E. Wilder, and R. A. Tobey. Flow cytometric localization within the cell cycle and isolation of viable cells following exposure to cytotoxic agents, *Cancer Research* **48**, 5742-5746 (1988).

D'Anna, J. A., and R. A. Tobey. Changes in nucleosome repeat lengths precede replication in the early-replicating metallothionein II gene region of cells synchronized in early S phase, *Biochemistry*, in press.

Delhaize, E., P. J. Jackson, L. D. Lujan, and N. J. Robinson. Poly(γ-glutamyl-cysteinyl)glycine synthesis in *Datura innoxia* and binding with cadmium: Role in cadmium tolerance, *Plant Physiol.*, in press.

Dethloff, L. A., and B. E. Lehnert. Pulmonary interstitial macrophages: Isolation and flow cytometric comparisons with alveolar macrophages and blood monocytes, *J. Leuk. Biol.* **43**, 80-90 (1988).

Doermann, A. H., A. Pao, and P. J. Jackson. Genetic control of capsid length in bacteriophage T4: Clustering of *ptg* mutations in gene 23, *J. Virology* **61**, 2823-2827 (1987).

Edwards, E. L., R. L. Ratliff, and D. M. Gray. Circular dichroism spectra of DNA oligomers show that short interior stretches of C·C+ base pairs do not form in duplexes with A·T base pairs, *Biochemistry* **27**, 5166-5174 (1988).

Elias, L., and H. A. Crissman. Interferon effects upon the adenocarcinoma 38 and HL-60 cell lines: antiproliferative responses and synergistic interactions with halogenated pyrimidine antimetabolites, *Cancer Research* **48**, 4868-4873 (1988).

Fowler, J. C. Modulation of neuronal excitability by endogenous adenosine in the absence of synaptic transmission, *Brain Research* **463**, 368-373 (1988).

Fowler, J. C., and J. M. O'Donnell. Antagonism of the responses to isoproterenol in the rat hippocampal slice with subtype-selective antagonists, *Eur. J. Pharmacology* **153**, 105-110 (1988).

Freyer, J. P. Role of necrosis in regulating the growth saturation of multicellular spheroids, *Cancer Research* **48**, 2432-2439 (1988).

Freyer, J. P., and P. L. Schor. Automated selective dissociation of cells from different regions of multicellular spheroids, *In Vitro Cell. Develop. Biol.*, in press.

Freyer, J. P., and P. L. Schor. Regrowth kinetics of cells from different regions of multicellular tumor spheroids of four cell lines, *J. of Cell. Physiol.*, in press.

Freyer, J. P., P. L. Schor, and A. G. Saponara. Partial purification of a protein growth inhibitor from multicellular spheroids, *Biochem. Biophys. Res. Comm.* **152**. 463-468 (1988).

Freyer, J. P., M. E. Wilder, P. L. Schor, J. R. Coulter, and M. R. Raju. A simple electronic volume cell sorter for clonogenicity assays, *Cytometry*, in press.

Gurley, L. R., J. G. Valdez, J. E. London, L. A. Dethloff, and B. E. Lehnert. An HPLC procedure for the analysis of proteins in lung lavage fluid, *Analytical Biochem.* **172**, 465-478 (1988).

Halliday, K., C. Fenoglio-Preiser and L. O. Sillerud. Differentiation of human tumors from non-malignant tissue by natural abundance carbon-13 NMR spectroscopy, *Magnetic Resonance in Medicine* **7**, 384-411 (1988).

Halliday, K., C. Fenoglio-Preiser, and L. O. Sillerud. Magnetic resonance spectroscopy of human tumors, *Journal of the College of American Pathology*, in press.

Hei, T. K., D. J. Chen, D. J. Brenner, and E. J. Hall. Mutations induced by charged particles of defined linear energy transfer, *Carcinogenesis* **9**, 1233-1236 (1988).

Heidorn, D. B., and J. Trewhella. Comparison of the crystal and solution structures of calmodulin and troponin C, *Biochemistry* **27**, 909-915 (1988).

Heyser, J. W., D. de Bruin, M. L. Kincaid, R. Y. Johnson, M. M. Rodriguez, and N. J. Robinson. Inhibition of NaCl-induced proline biosynthesis by exogenous proline in halophilic *Distichlis spicata* suspension cultures, *J. Exp. Botany*, in press.

Hjeresen, D. L., D. J. Brief, D. L. Amend, D. M. Dorsa, and S. C. Woods. The role of arginine vasopressin in the development of tolerance to ethanol in normal and Brattleboro rats, *Peptides* **9** (Suppl. 1) 193-200 (1988).

Hjeresen, D. L., and J. Diaz. Ontogeny of susceptibility to experimental febrile seizures in rats, *Developmental Psychobiology* **21**, 261-275 (1988).

Hjeresen, D. L., A. Francendese, and J. M. O'Donnell. Microwave interaction with ethanol hypothermia: Tolerance, time course exposure, duration and ethanol dose studies, *Bioelectromagnetics* **9**, 63-78 (1988).

Johnston, R. G., S. B. Singham, and G. C. Salzman. Polarized light scattering, *Comments on Mol. Cell. Biophys.* **5**, 171 (1988).

Johnston, R. G. Rapid, differential micro-thermometry using Zeeman interferometry, *Applied Physics Letters*, in press.

Kowluru, R. A., D. B. Heidorn, S. P. Edmondson, M. W. Bitensky, A. Kowluru, N. W. Downer, T. W. Whaley, and J. Trewhella. Glycation of calmodulin: chemistry, structural and functional consequences, *Biochemistry*, in press.

Kowluru, R. A., M. W. Bitensky, A. Kowluru, P. A. Keaton, M. Dembo, and T. Buican. Reversible sodium pump defect and swelling in the diabetic red cell: effects on filterability and pathological implications for the microcirculation, *Proc. Natl. Acad. Sci* (U.S.A.), in press.

Krolewski, B., J. B. Little, and R. J. Reynolds. Effect of duration of exposure to benzo(a)pyrene diol-epoxide on neoplastic transformation, mutagenesis, cytotoxicity and total covalent binding to DNA of rodent cells, *Teratogenesis, Carcinogenesis and Mutagenesis* **8**, 127-136 (1988).

Lehnert, B. E., and C. Sanz-Rodriguez. Expression of Fcγ receptor-mediated phagocytosis by airway intra-luminal macrophages, *Immunol. Invest.* **17**, 225-235 (1988).

Lehnert, B. E., Y. E. Valdez, and L. A. Dethloff. Leukocytic responses to the intrapleural deposition of particles, particle-cell associations, and the clearance of particles from the pleural space compartment, *J. Toxicol. Environ. Health* **24**, 41-66 (1988).

Li, S. L., M. L. Luedemann, F. S. Sharief, I. Sakai, T. Takao, and L. L. Deaven. Mapping of human lactate dehydrogenase-A, B and C genes and pseudogenes: LDH-C gene is located with LDH-A gene on chromosome 11, not with LDH-B gene on chromosome 12, *Cytogenetics and Cell Genetics* **48**, 10-18 (1988).

Lin, P. H., R. H. Selinfreund, E. Walshull, and W. Wharton. Rapid isolation of plasmalemma from cultured A431 cells: Characterization of epidermal growth factor receptors, *Analytical Biochem.* **168**, 300-305 (1988).

Logan, K. A., M. E. Dahmus, and E. M. Bradbury. Histone H1° shares epitopes with RNA polymerase II: Implications in development and aging, *Journal of Biological Chemistry* **263**, 9658-9662 (1988).

Longmire, J. L., and A. K. Lewis. DNA fingerprinting and sex linkage of M13-complementary sequences in endangered falcons, *Journal of Heredity*, in press.

Longmire, J. L., A. K. Lewis, N. C. Brown, J. M. Buckingham, L. M. Clark, M. D. Jones, L. J. Meincke, J. Meyne, R. L. Ratliff, F. A. Ray, R. P. Wagner, and R. K. Moyzis. Isolation and molecular characterization of a highly polymorphic centromeric tandem repeat in the family *Falconidae*, *Genomics* **2**, 14-24 (1988).

Marrone, B. L., and E. K. Asem. Cyclic AMP production in avian theca cells during follicular maturation, *J. Reprod. Fert.* **83**, 67-71 (1988).

Marrone, B. L., and H. A. Crissman. Characterization of the granulosa cell subpopulations from avian preovulatory follicles by multiparameter flow cytometry, *Endocrinology* **122**, 651-658 (1988).

Marrone, B. L., and R. J. Sebring. Quantitative cytochemistry of 3β-hydroxysteroid dehydrogenase activity in avian granulosa cells during follicular maturation, *Biology of Reproduction*, in press.

McElroy, J. F., and J. M. O'Donnell. Discriminative stimulus effects of haloperidol: Evidence for dopaminergic mediation, *Drug Dev. Res.*, in press.

McElroy, J. F., and J. M. O'Donnell. Discriminative stimulus properties of clenbuterol: Evidence for beta adrenergic involvement, *J. Pharmocol. Exp. Ther.* **245**, 155-163 (1988).

McElroy, J. F., J. J. Stimmel, and J. M. O'Donnell. Effects of centrally acting beta adrenergic agonist on discrete trial conditioned avoidance behavior in rats, *Psychopharmacology*, in press.

Moyzis, R. K., J. M. Buckingham, L. S. Cram, M. Dani, L. L. Deaven, M. D. Jones, J. Meyne, R. L. Ratliff, and J. R. Wu. A highly conserved repetitive DNA sequence (TTAGGG) is present at the telomeres of human chromosomes, *Proc. Natl. Acad. Sci.* **85**, 6622-6626 (1988).

Nagasawa, H., D. J. Chen, and G. F. Strniste. Response of x-ray sensitive CHO mutant cells to γ-irradiation: I. Effects of low-dose rates and the process of PLD repair in G_1-phase, *Radiat. Res.*, in press.

Nierman, W. C., C. Trypus, and L. L. Deaven. Preservation and stability of bacteriophage lambda libraries by freezing in liquid nitrogen, *Biotechniques* **5,** 724-728 (1988).

O'Donnell, J. M. Behavioral consequences of activation of beta adrenergic receptors by clenbuterol: Evidence for mediation by the central nervous system, *Brain Res. Bull.*, in press.

O'Donnell, J. M. Psychopharmacological consequences of activation of beta adrenergic receptors by SOM-1122, *J. Phamacol. Exp. Ther.* **246**, 38-46 (1988).

Okinaka, R. T., T. W. Whaley, and G. F. Strniste. Differential effects induced by N-hydroxy-2-acetylaminofluorene and N-hydroxy-2-aminofluorene in DNA excision repair-deficient Chinese hamster cells, *Mutation Res.* **209,** 45-50 (1988).

Radinsky, R., P. M. Kraemer, M. R. Proffitt, and L. A. Culp. Clonal diversity of the kirsten-ras oncogene during tumor progression in athymic nude mice: mechanisms of amplification and rearrangement, *Cancer Research* **48,** 4941-4953 (1988).

Raju, M. R., and A. J. van der Kogel. Radiobiology of pions in comparison with other heavy particles, *Radiotherapy and Oncology*, in press.

Roberge, M., M. E. Dahmus, and E. M. Bradbury. Chromosomal loop/nuclear matrix organization of transcriptionally active and inactive RNA polymerases in HeLa nuclei, *Journal of Molecular Biology* **201,** 545-555 (1988).

Roberge, M. and E. M. Bradbury. Nascent RNA contacts the two large polymerase subunits and a 52 kDa polypeptide in RNA polymerase I transcribing complexes, *Journal of Biological Chemistry*, in press.

Robinson, N. J., R. L. Ratliff, P. J. Anderson, E. Delhaize, J. M. Berger, and P. J. Jackson. Biosynthesis of poly(γ-glutamylcysteinyl)glycines in cadmium-tolerant *Datura innoxia* (Mill.) cells, *Plant Sci.* **56,** 197-204 (1988).

Rodriguez, M.M., J.W. Heyser. Growth inhibition by exogenous proline and its metabolism in saltgrass *(Distichlis spicata)* suspension cultures, *Plant Cell Reports* **7**, 305-308 (1988).

Salzman, G. C., R. B. Krall, and J. G. Marinuzzi. Knowledge-engineering software. A demonstration of a high-end tool, *Anal. and Quant. Cyt. Hist.* **10**, 219-224 (1988).

Schillaci, M. E., S. Carpenter, M. R. Raju, R. J. Sebring, M. E. Wilder, and D. T. Goodhead. Radiobiology of ultrasoft x rays. II. Cultured C3H mouse cells (10T1/2), *Radiation Research*, in press.

Schwarzacher-Robinson, T., L. S. Cram, J. Meyne, and R. K. Moyzis. Characterization of human heterochromatin by *in situ* hybridization with satellite DNA clones, *Cyto. and Cell Genetics* **47,** 192-196 (1988).

Schwarzacher-Robinson, T., P. M. Kraemer, and L. S. Cram. Spontaneous *in vitro* neoplastic evolution: non-activity of cultured Chinese hamster cells, *Cancer Genetics and Cytogenetics*, in press.

Selinfreund, R. H., P. H. Lin, J. L. Cooper, and W. Wharton. Multiple effects of phenothiazines on the binding and processing of epidermal growth factor by BALB/c-3T3 cells, *Amer. J. Physiol.* **251**, 904-911 (1988).

Sillerud, L. O., J. P. Freyer, and M. A. Mattingly. Proton NMR microscopy of multicellular tumor spheroid microphysiology, *J. Mag. Reson. Med.*, in press.

Sillerud, L. O., K. R. Halliday, R. H. Griffey, C. Fenoglio-Preiser, and S. Sheppard. *In vivo* ^{13}C NMR spectroscopy of the human prostate, *Mag. Reson. in Med.* **8**, 224-230 (1988).

Sillerud, L. O., D. B. van Hulsteyn, and R. H. Griffey. {^{13}C}-Polarization transfer proton NMR imaging of a sodium [^{13}C]formate phantom at 4.7 Tesla, *J. Magn. Res.* **76**, 380-385 (1988).

Sinex, D. G., and L. P. McDonald. Average discharge rate representation of voice-onset time in the chinchilla auditory nerve, *J. Acoust. Soc. Am.* **83**, 1817-1827 (1988).

Sinex, D.G., and L. P. McDonald. Synchronized discharge rate representation of voice-onset time in the chinchilla auditory nerve, *J. Acoust. Soc. Am.*, in press.

Singham, S. B., and C. F. Bohren. A hybrid method in light scattering by an arbitrary particle, *Appl. Opt.*, in press.

Singham, S. B., and C. F. Bohren. Light scattering by arbitrary particles: The scattering order formulation of the coupled dipole method, *J. Opt. Soc. Amer.* **5**, 1867-1872 (1988).

Smyth, M. J., T. G. O'Brien, and W. R. Wharton. Identification of a variant subclone of BALB/c-3T3 cells with an altered mitogenic requirement for insulin, *Journal of Cellular Physiology,* in press.

Stavert, D. M., and B. E. Lehnert. Cross-ventilating rat model: Pulmonary functional status of participating animals, *J. Aerosol Med: Deposition, Clearance, and Effects in the Lung* **1**, 51-66 (1988).

Stavert, D. M., and B. E. Lehnert. Potentiation of the expression of nitrogen dioxide-induced lung injury by post exposure exercise, *Environ. Res.*, in press.

Stewart, C. C., A. P. Stevenson, and R. C. Habbersett. The effect of low dose irradiation on resting and PHA stimulated human lymphocyte subsets, *Intl. J. of Rad. Biol.* **53**, 77-87 (1988).

Tobey, R. A., N. Oishi, and H. A. Crissman. Synchronized human diploid fibroblasts: Progression capabilities of a subpopulation that fails to keep pace with the predominant, rapidly-dividing cohort of cells, *J. Cell. Physiol.*, in press.

Tobey, R. A., J. G. Valdez, and H. A. Crissman. Synchronization of human diploid fibroblasts at multiple stages of the cell cycle, *Exp. Cell Res.*, in press.

Tomei, F. A., D. Rouse, J. Maki, and R. Mitchell. Presence of an unusual methanogen in coal gasification waste, *Appl. Environ. Microbiol.*, in press.

Traganos, F., H. A. Crissman, and Z. Darzynkiewicz. Staining with pyronin Y detects changes in conformation of RNA during mitosis and hyperthermia of CHO cells, *Experimental Cell Research*, in press.

Trewhella, J., V. A. P. Carlson, E. H. Curtis, and D. B. Heidorn. Differences in the solution structures of oxidized and reduced cytochrome c using small angle x-ray scattering, *Biochemistry* **27**, 1121-1125 (1988).

Trewhella, J., D. B. Heidorn, and P. A. Seeger. Solution structures of calcium regulating proteins: A small-angle scattering study, *Molecular Crystals and Liquid Crystals*, in press.

Trewhella, J., W. K. Liddle, D. B. Heidorn, and N. Strynadka. Calmodulin and troponin C structures studied by Fourier Transform Infrared Spectroscopy: Effects of Ca^{2+} and Mg^{2+} binding, *Biochemistry*, in press.

Valdez, Y. E., and B. E. Lehnert. A procedure for instilling agents into the pleural space compartment of the rat without co-administration into the lung compartment, *Animal Technol.* **39**, 1-8 (1988).

van der Kogel, A. J., K. A. Jarett, M. A. Paciotte, and M. R. Raju. Radiation tolerance of the rat rectum to fractionated x-rays and Pi mesons, *Radiotherapy and Oncology*, in press.

van der Kogel, A. J., J. C. Martin, A. Smith, and M. R. Raju. Late effects of fractionated PI-meson compared to x-rays on mouse lung, *International J. of Rad. Oncology, Biol., and Physics*, in press.

van der Kogel, A. J., and M. R. Raju. Tolerance of spinal cord, lung, and rectum after fractionated pions and x-rays, *Radiotherapy and Oncology*, in press.

Varghese, S., L. L. Deaven, Y.-C. Huang, R. K. Gill, A. M. Iacopino, and S. Christakos. Transcriptional regulation and chromosomal assignment of the mammalian calbindin-D_{28k} gene, *Cytogenetics and Cell Genetics*, in press.

Voelz, G. L., A. P. Stevenson, and C. C. Stewart. Does plutonium intake in workers affect lymphocyte function? *Radiation Protection Dosimetry*, in press.

REVIEW ARTICLES

Cram, L. S., M. F Bartholdi, F. A. Ray, M. C. Habbersett, and P. M. Kraemer. Univariate flow karyotype analysis, in *Flow Cytogenetics*, J. W. Gray, Ed. (Academic Press, Inc.), in press.

Cram, L. S., M. F. Bartholdi, F. A. Ray, J. Meyne, R. K. Moyzis, T. Schwarzacher-Robinson, and P. M. Kraemer. Overview of flow cytogenetics for clinical applications, in *Cytometry* (Alan R. Liss, Inc., New York), in press.

Crissman, H. A., and J.A. Steinkamp. Cytochemical techniques for multivariate analysis of DNA and other cellular constituents, in *Flow Cytometry and Sorting*, M. Melamed, T. Lindmo, and M. Mendelsohn, Eds. (John Wiley and Sons, New York), in press.

Gray, J. W., and L.S. Cram. Flow karyotyping and chromosomes sorting, in *Flow Cytometry and Sorting*, M. Melamed, T. Lindmo, and M. Mendelsohn, Eds. (John Wiley and Sons, New York), in press.

Gurley, L. R., J. E. London, L. A. Dethloff, D. M. Stavert, and B. E. Lehnert. Analysis of proteins in bronchoalveolar lavage fluids during pulmonary edema resulting from nitrogen dioxide and cadmium exposure, in *Techniques in Protein Chemistry*. T. Hugli, Ed. (Academic Press, Inc.), in press.

Gurley, L. R., W. D. Spall, J. G. Valdez, P. S. Jackson, D. A. Prentice, J. Meyne, and F. A. Ray. HPLC of histones, in *Methods and Applications*, K. M. Gooding and F. Regnier, Eds. (Marcel Dekker, New York), in press.

Jackson, P. J., N. J. Robinson, and E. Delhaize. Cd-tolerance in plant cells: A comparison of biochemical and molecular properties of tolerant and sensitive cells. in *Metal Ion Homeostasis: Molecular Biology and Chemistry,* D. Winge and D. Hamer, Eds., Vol. 98 (Alan R. Liss, Inc., New York), in press.

Johnston, R. J., S. P. Edmondson, S. B. Singham, and G. C. Salzman. Biophysical applications of the XUV free electron laser, in *Technical Digest, Free Electron Laser Applications in the Ultraviolet* (Optical Society of America, Washington, D.C.) 126-128 (1988).

Lehnert, B. E., Y. E. Valdez, and S. H. Bomalaski. Analyses of particles in the lung free cell, tracheobronchial lymph nodal, and pleural space compartments following their deposition in the lung as related to lung clearance mechanisms, in *Inhaled Particles* VI, J. Dodgson, R.I. McCallum, Eds., in press.

Longmire, J. L. Identification and development of breeding population-specific DNA polymorphisms in the genome of the Peregrine Falcon, in *Peregrine Falcon Populations, Their Management and Recovery*, T. Cade, Ed., Peregrine Fund, Inc., Boise, ID (1988).

Lucas, J., and M. F. Bartholdi. Chromosome classification by scanning flow cytometry, in *Flow Cytogenetics*, J. Gray, Ed. (Academic Press, Orlando, FL), in press.

Reynolds, R.J., and L.H. Lam. Measurement of closely opposed pyrimidine dimers as bifilar enzyme-sensitive sites, in *Handbook of DNA Repair Techniques.* E.C. Friedberg and P.C. Hanawalt, Eds., Vol. III, (Marcel Dekker, Inc., NY), 427-442 (1988).

Salzman, G. C., S. B. Singham, R. G. Johnston, and C. F. Bohren. Light scattering and flow cytometry, in *Flow Cytometry and Sorting,* M. R. Melamed, T. Lindmo, and M. L. Mendelsohn, Eds. (Alan R. Liss, New York), in press.

Stewart, C. C., A. P. Stevenson, and J. Hibbs. Effector mechanisms for macrophage -induced cytostasis and cytolysis of tumor cells, in *Macrophages and Cancer* (CRC Press, Boca Raton, FL), in press.

Wharton, W., and M. J. Smyth. Growth and maintenance of BALB/c-3T3 cells, in *Cell Growth and Division: A Practical Approach*, R. Baserga, Ed. (IRL Press, Oxford), in press.

Yamazaki, A., M. Tatsumi, and M.W. Bitensky. Purification of rod outer segment GTP-binding protein subunits and cGMP phosphodiesterase by single-step column chromatography, *Methods of Enzymology* **159,** 702-710 (1988).

Yamazaki, A., M.W. Bitensky, and J.E. Casnellie. Photoaffinity labeling of high-affinity cGMP-specific noncatalytic binding sites on cGMP phosphodiesterase of rod outer segments, *Methods of Enzymology* **159,** 730-736 (1988).

CONFERENCE PROCEEDINGS AND REPORTS

Buican, T. N. Microrobotics for cell biology research, in *Technology '88: U.S. Department of Energy R&D Laboratory Technology Transfer Program*, in press.

D'Anna, J. A., D. L. Grady, and R. A. Tobey. Chromatin structural changes precede replication in initiated replicons during inhibition of DNA elongation, in *Mechanisms and Consequences of DNA Damage Processing, UCLA Symposia in Molecular and Cellular Biology*, New Series, E. C. Friedberg and P. C. Hanawalt, Eds. (Alan R. Liss Inc., New York), **Vol. 83**, 539-543 (1988).

Deaven, L. L., and M. A. Van Dilla. The National Laboratory Gene Library Project in *Proceedings of the 27th Hanford Symposium on Health and Environment*, in press.

Gurley, L. R., Y. E. Valdez, J. G. London, W. D. Spall, L. A. Dethloff, and B. E. Lehnert. Development and characterization of an HPLC system for the fractionation of bronchoalveolar lavage fluid, Los Alamos National Laboratory report LA-11309-MS, (1988).

Hildebrand, C. E., R. L. Stallings, L. L. Deaven, J. L. Longmire, L. S. Cram, J. Meyne, R. K. Moyzis, and D. Callen. Physical maps of human chromosome 16: Status and perspectives, Genome Mapping and Sequencing Meeting, Cold Spring Harbor, NY, April 27-May 1 (1988).

Javel, E., J. B. Mott, N. Rush, and D. W. Smith. Frequency discrimination: Evaluation of rate and temporal codes, in *Basic Issues in Hearing - The 8th International* Symposium, H. Duifhuis, J.W. Horst and H.P. Wit, Eds. Academic Press, London (1988).

Johnston, R. G. and G. C. Salzman. Phase differential scattering and polydispersity, in *Proceedings, 1988 CRDEC Conference on Obscuration and Aerosol Research*, in press.

Lehnert, B. E. Pulmonary pathophysiology of oxides of nitrogen, and pulmonary pathophysiology of halide gases, Military Medical Pulmonary Research Review and Analysis, Ft. Fitzsimmons, Denver, CO., September 20-22 (1988).

Lehnert, B. E. Airway intra-luminal macrophages: Potential roles in defense, injury, and repair of airway epithelium, Biology, Toxicology, and Carcinogenesis of Respiratory Epithelium, Lovelace Inhalation Toxicology Research Insititute, Albuquerque, N.M., November 14-16 (1988).

Lehnert, B. E., K. E. Toevs, Y. E. Valdez, and R. J. Sebring. Particle-macrophage relationships during the clearance of particles from the alveolar macrophage compartment. Los Alamos National Laboratory report LA-11428-MS (1988).

London, J. E. A toxicological study of gadolinium nitrate, Los Alamos National Laboratory report LA-11261-MS (1988).

London, J. E. Toxicological study of yttrium barium copper oxide, Los Alamos National Laboratory report LA-11437-MS (1988).

London, J. E. A toxicological study of 1,2,4-Triazole-5-One, Los Alamos National Laboratory report, in press.

Norton, S. J., J. B. Mott, and S. T. Neely. Changes in spontaneous otoacoustic emissions produced by acoustic stimulation, in *Basic Issues in Hearing, 8th International Symposium*, H. Diufhuis, J.W. Horst, and H.P. Wit, Eds., Academic Press, London (1988).

Okinaka, R. T., and G. F. Strniste. Mutations induced at the HPRT locus in a DNA excision repair deficient Chinese hamster cell line after treatment with an acetamido-PAH, in *Polynuclear Aromatic Hydrocarbons*, Vol. XI, in press.

Okinaka, R. T., T. W. Whaley, U. Hollstein, J. W. Nickols, and G. F. Strniste. Identification of a promutagenic compound formed by the action of near ultraviolet light on a primary aromatic amine, in *Polynuclear Aromatic Hydrocarbons: A Decade of Research*, Vol. X, M. Cooke and A. J. Dennis, Eds., Battelle Press, Columbus, OH pp. 661-671 (1988).

Reynolds, R. J. Repair of closely opposed pyrimidine dimers in UV-irradiated Chinese hamster ovary cells. 1988 UCLA Symposium on Mechanisms and Consequences of DNA Damage Processing, Taos, New Mexico, January 24-30 (1988).

Serden, L. S., G. A. Marek, J. M. O'Donnell, A. Li, R. Dunn, and D. Jolly. The role of noradrenergic and serotonergic systems in the screening of antidepressant drugs, in *Proceedings of the Sixth International Catecholamine Symp.* (Alan R. Liss Inc., New York), in Press.

Strniste, G. F., D. J. Chen, D. de Bruin, J. A. Luke, L. S. McCoy, J. S. Mudgett, J. W. Nickols, R. T. Okinaka, J. G. Tesmer, and M. A. MacInnes. Restoration of Chinese hamster cell radiation resistance by the human repair gene ERCC-5 and progress in molecular cloning of this gene, in *Mechanisms and Consequences of DNA Damage Processing*, UCLA Symposium on Molecular and Cellular Biology, New Series, E. Friedberg and P. Hanawalt, Eds., (Alan R. Liss, Inc., New York), **Vol. 83**, 301-306 (1988).

Wrobleski, D., D. Cash, D. Duchane, B. E. Lehnert, J. E. London, and R. Kossowsky. Surface treatment of PMMA with titanium and polyvinylpyrrolidone and its effect on blood compatibility, in *Applied Bioactive Polymeric Systems*, Plenum Publ. Corp., in press.

ABSTRACTS

Aine, C. J., J. S. George, M. T. Oakley, P. A. Medvick, and E. R. Flynn. Source localization of components of the visual-evoked neuromagnetic response, Society for Neuroscience Abstracts, p. 750 (1988).

Albright, K. L., J. C. Martin, and L. S. Cram. High speed chromosome sorting, 13th International Meeting of the Society for Analytical Cytology, Breckenridge, CO, September 4-9 (1988).

Bartholdi, M. F. Analysis and sorting of human chromosomes on an EPICS V flow cytometer, EPICS Users Meeting, New York, NY, June (1988).

Bartholdi, M. F., J. Meyne, and L. S. Cram. Chromosome banding analysis by slit scan flow cytometry, 13th International Meeting of the Society for Analytical Cytology, Breckenridge, CO, September 4-9 (1988).

Behr, M. J., D. F. Kusewitt, D. M. Stavert, and B. E. Lehnert. Comparisons of nitrogen dioxide (NO_2) and nitric oxide (NO) as inducers of acute pulmonary injury when inhaled at relatively high concentrations for brief periods. *The Toxicologist* **8**(1), p. 557A (1988).

Bell-Prince, C., M. C. Habbersett, and L. S. Cram. National flow cytometry resource, 13th International Meeting of the Society for Analytical Cytology, Breckenridge, CO, September 4-9 (1988).

Bentley, B. F., J. R. Lopez, and J. C. Martin. Improved real time sorting delay monitors, 13th International Meeting of the Society for Analytical Cytology, Breckenridge, CO, September 4-9 (1988).

Buican, T. N. Dynamic interpretation of immunofluorescence flow cytometric data, 13th International Meeting of the Society for Analytical Cytology, Breckenridge, CO, September 4-9 (1988).

Buican, T. N. Single cell manipulation by optical trapping, 3rd Annual Symposium, Southwest Consortium on Plant Genetics and Water Resources, Las Cruces, NM, April 18-19 (1988).

Buican, T. N., D. L. Neagley, and W. C. Morrison. Ocam - A robotic optical trapping cell analyzer and manipulator, 13th International Meeting of the Society for Analytical Cytology, Breckenridge, CO, September 4-9 (1988).

Chen, D. J., H. Nagasawa, J. B. Little, and G. F. Strniste. Effects of low-dose rate γ-ray radiation and dose fractionation on x-ray sensitive Chinese hamster (CHO) cell mutants, 36th Annual Meeting of the Radiation Research Society, **#Cp-2** (1988).

Clark, L., R. Moyzis, R. Stallings, J. Longmire, L. Deaven, J. Fawcett, K. Albright, M. Leudemann, J. Meyne, D. Callen, and C. Hildebrand. Long-range mapping around human centromeric repetitive sequence clusters using flow-sorted chromosomes and macrorestriction analysis, 16th International Congress of Genetics, Toronto, Canada, *Genome 30*, Suppl. 1, 245 (1988).

Cornforth, M. N. Radiation-induced damage and repair, as measured by premature chromosome condensation, 36th Annual Meeting of the Radiation Research Society, Philadelphia, PA, April 16-21 (1988).

Cornforth, M. N., M. E. Schillaci, and M. R. Raju. Small volume ($\leq$ 7nm) energy depositions effective in producing isochromatid aberrations in CHO cells, 36th Annual Meeting of the Radiation Research Society, Philadelphia, PA, April 16-21 (1988).

Crissman, H. A., N. Oishi, and J. A. Steinkamp. Analysis of DNA replication patterns in BrdU-labeled cell populations and conditions for correlated measurement of RNA and FITC-labeled components, 13th International Meeting of the Society for Analytical Cytology, Breckenridge, CO, September 4-9 (1988).

Crissman, H. A., and J. A. Steinkamp. Correlated flow cytometric (FCM) analyses of cell cycle, DNA replicative potential and cell metabolism in differentiating mammalian cells, 12th Annual Meeting of the Cell Kinetics Society, Omaha, NE, March 24-27 (1988).

Crowell, R., B. E. Lehnert, and C. Mold. Identification of pulmonary macrophage populations in the mouse, Twenty-fifth National Meeting of the Society for Leukocyte Biology, Washington, DC, October 27-30, 1988, *J. Leuk. Biol.* **44**, 74A (1988).

D'Anna, J. A., D. L. Grady, and R. A. Tobey. Chromatin structural changes precede replication in initiated replicons during inhibition of DNA elongation, *J. Cell Biochem.*, **Supplement 12A,** 355 (1988).

Deaven, L. L., and M. A. Van Dilla. The National Laboratory Gene Library Project, Hanford Life Sciences Symposium, Richland, WA, October 18-20 (1988).

Deaven, L. L., and M. A. Van Dilla. The National Laboratory Gene Library Project, 13th International Meeting of the Society for Analytical Cytology, Breckenridge, CO, September 4-9 (1988).

Delhaize, E., N. J. Robinson, and P. J. Jackson. The effect of cadmium on gene expression in cadmium-tolerant and cadmium-sensitive *Datura innoxia* cells, American Association of Plant Physiologists Annual Meeting, Reno NV, July 10-14 (1988).

Freyer, J. P., P. L. Schor, and L. O. Sillerud. Noninvasive NMR spectroscopy and imaging of conditions inside intact multicellular tumor spheroids, 36th Annual Meeting of the Radiation Research Society, Philadelphia, PA, April 16-21 (1988).

George, J. S., C. J. Aine, and E. R. Flynn. Neuromagnetic visual evoked responses to sinusoidal gratings, Society for Neuroscience Abstracts, p. 1012 (1988).

George, J. S., C. J. Aine, and E. R. Flynn. Neuromagnetic source localization of components of the visual evoked response, Biophysical Society, in press.

Grady, D. L., R. L. Ratliff, D. L. Robinson, and R. K. Moyzis. Binding of HeLa cell nuclear proteins to human centromeric and telomeric repetitive DNA sequences. *American J. of Human Genetics* **43**, A185 (1988).

Gurley, L. R., J. E. London, L. A. Dethloff, A. J. van der Kogel, and B. E. Lehnert. Use of HPLC to measure lung injury following biological, chemical, and physical insults, 14th International Congress of Biochemistry, Prague, Czechoslovakia, July 10-15 (1988).

Gurley, L. R., J. E. London, L. A. Dethloff, D. M. Stavert, and B. E. Lehnert. Analysis of proteins in bronchoalveolar lavage fluids during pulmonary edema resulting from nitrogen dioxide and cadmium exposure, Second Symposium of the Protein Society, San Diego, CA, August 13-17 (1988).

Halliday, K. R., D. D. Mickey, C. Fenoglio-Preiser, J. Hutson, N. Fink, and L. O. Sillerud. *In vitro* ^{13}C NMR Spectroscopy of Dunning Rat Prostatic Tumors, *Proc. Soc. Magn. Reson. Med.* 7 494 (1988).

Heyser, J. W., Z. Zhao, and H. J. Bohnert. Isolation and characterization of proteins and mRNAs regulated by NaCl from halophilic *Distichlis spicata* suspension cultures. Invited talk at 2nd International Congress of Plant Molecular Biology, Jerusalem, Israel, November 13-18 (1988).

Heyser, J. W., Z. Zhao, and H. J. Bohnert. Characterization of mRNAs and proteins induced by NaCl in *Distichlis spicata* suspensions. Invited talk at 3rd Annual Symposium, Southwest Consortium on Plant Genetics and Water Resources, Las Cruces, NM, April 18-19 (1988).

Hildebrand, C. E., L. M. Clark, J. Meyne, J. M. Buckingham, M. D. Jones, N. C. Brown, J. L. Longmire, L. L. Deaven, D. Callen, R. L. Stallings, and R. K. Moyzis. Accelerated physical mapping of the human genome: macrorestriction analysis of centromeric and telomeric regions, *Am. J. Human Genet. 43*, A147 (1988).

Hildebrand, C. E., R. K. Moyzis, L. L. Deaven, J. L. Longmire, K. Sirotkin, W. B. Goad, and R. L. Stallings. Human chromosome - specific physical mapping: theoretical and experimental approaches and applications, Hanford Life Sciences Symposium, Richland, WA, October 18-20 (1988).

Hildebrand, C. E., R. L. Stallings, L. L. Deaven, L. S. Cram, M. F. Bartholdi, J. L. Longmire, J. Meyne, J. H. Jett, R. L. Ratliff, T. G. Marr, K. M. Sirotkin, W. B. Goad, G. A. Evans, and R. K. Moyzis. Long-range physical mapping and cloning of human chromosomes, 13th International Meeting of the Society for Analytical Cytology, Breckenridge, CO, September 4-9 (1988).

Hjeresen, D.L. Effects of cocaine on acquisition and retention of ethanol tolerance, Neurosci. Abst. **14**, 766 (1988).

Hudson, C., R. A. Sneed, and A. P. Stevenson. The expansion of murine splenocytes in interleukin-2, MARC Scholars Conference and Program Directors Meeting, Bethesda, MD, November 2-5 (1988).

Jackson, P. J., N. J. Robinson, and E. Delhaize. Cd-tolerance in plant cells: A comparison of biochemical and molecular properties of tolerant and sensitive cells, UCLA Symposium: Metal Ion Homeostasis: Molecular Biology and Chemistry, Frisco, CO, April 10-16 (1988).

Jackson, P. J., N. J. Robinson, and E. Delhaize. Plant strategies and genetic resources for trace metal tolerance. Third Annual Symposium of the Southwest Consortium on Plant Genetics and Water Resources. Las Cruces, NM April 18-19 (1988).

Jackson, P. J., E. Delhaize, L. A. Harrison, and N. J. Robinson. Mechanisms of trace metal-tolerance in plants, The Third Chemical Congress of North America, Toronto, Ontario, Canada, June 6-10 (1988).

Jett, J. H. Flow cytometry. Where is it headed? 13th International Meeting of the Society for Analytical Cytology, Breckenridge, CO, September, 4-9 (1988).

Keng, P. C., P. A. Jeggo, and D. J. Chen. Age responses of Chinese hamster ovary x-ray-sensitive mutant cells xrs-5 and xrs-6, 36th Annual Meeting of the Radiation Research Society, **#Dk-9** (1988).

Kraemer, P. M. Genetic and cytogenetic instability: primary driver of the neoplastic process, Hanford Life Sciences Symposium, Richland, WA, October 18-20 (1988).

Kraemer, P. M., M. F. Bartholdi, R. L. Ratliff, and J. L. Longmire. Application of flow sorted chromosomes in cancer research, 13th International Meeting of the Society for Analytical Cytology, Breckenridge, CO, September 4-9 (1988).

Kraemer, P. M., R. L. Ratliff, M. F. Bartholdi, and J. L. Longmire. Use of VNTR (minisatellite) sequence for monitoring chromosomal instability, Gatlinburg Symposium, Gatlinburg, TN, April 11-14 (1988).

Krall, R., J. D. Parson, and G. C. Salzman. Device independent graphics for cytometry data, 13th International Meeting of the Society for Analytical Cytology, Breckenridge, CO, September 4-9 (1988).

Kusewitt, D. F., D. M. Stavert, M. J. Behr, and B. E. Lehnert. Delayed nitrogen dioxide (NO_2)-associated type II cell hyperplasia with increasing concentrations of inhaled NO_2. *The Toxicologist* **8**(1), p 555A (1988).

Lehnert, B. E. Distributions of particles in alveolar macrophages during lung clearance, the Seventh International Congress on Aerosols in Medicine, Rochester, NY, September 25-29, 1988, *J. Aerosol Med.* **1**, p. 206 (1988).

Lehnert, B. E. Lung clearance and retention of particles: Mechanisms involving pulmonary macrophages, the Seventh International Congress on Aerosols in Medicine, Rochester, NY, September 25-29, *J. Aerosol Med.* **1**, p. 214 (1988).

Lehnert, B. E., and C. Sanz-Rodriguez. Expression of Fcγ receptor-mediated phagocytosis by airway intra-luminal macrophages compared to alveolar macrophages, Annual Meeting of the American Thoracic Society, Las Vegas, NV, May 8-11, *Am. Rev. Respir Dis.* **137**, p. 86A (1988).

Lehnert, B. E., K. E. Toevs, Y. E. Valdez, and R. J. Sebring. Particle-macrophage relationships during the clearance of particles from the alveolar macrophage compartment, *The Toxicologist* **8**(1), p. 276A (1988).

Lehnert, B. E., Y. E. Valdez, L. A. Dethloff, and E. A. Margiotta. Kinetics of bleomycin-induced cell population alterations in the alveolar space and pulmonary interstitial compartments, Annual Meeting of the American Thoracic Society, Las Vegas, NV, May 8-11, *Am. Rev. Respir. Dis.* **137**, p. 431A (1988).

Longmire, J. L., N. C. Brown, L. J. Meincke, L. Clark, M. L. Leudemann, K. L. Albright, J. J. Fawcett, E. W. Campbell, R. K. Moyzis, C. E. Hildebrand, and L. L. Deaven. High efficiency cloning of partial digest human chromosome-specific DNA, 16th International Congress of Genetics, Toronto, Canada, *Genome 30*, Suppl. 1, 245 (1988).

Longmire, J. L., N. C. Brown, L. J. Meincke, M. L. Luedemann, K. L. Albright, J. J. Fawcett, E. W. Campbell, R. K. Moyzis, C. E. Hildebrand, and L. L. Deaven. Construction, characterization, and applications of human chromosome 16-specific partial digest libraries, *Am. J. Human Genet.*, **Vol. 43**, No. 3, 597 (1988).

MacInnes, M. A., and J. S. Mudgett. CpG islands and associated CpG methylation in the 5' promoter regions of three human DNA repair genes, Nucleic Acid Methylation and Gene Expression, UCLA Symposium on Molecular and Cellular Biology (1988).

MacInnes, M. A., R. T. Okinaka, D. J. Chen, J. W. Nickols, J. G. Tesmer, L. S. McCoy, and G. F. Strniste. Molecular-genetic evidence for identification of the ERCC-5 human DNA excision repair gene, *J. Cell Biochem.*, **Suppl. 12A**, 319 (1988).

Marrone, B. L. Regional pattern of cell maturation in the hen granulosa layer. *Biol. Reprod.* **38**, Suppl. 1, p. 176 (1988).

Marrone, B. L., and R.J. Sebring. Changes in steroid enzyme activity during follicular maturation in avian granulosa cells as measured by image analysis of stained cells, *Endocrinology* **122**, Suppl. 1, p. 199 (1988).

Martin, J. C., J. R. Lopez, H. A. Crissman, and G. C. Saunders. Detection of luminescence from infrared fluorescent dyes. 13th International Meeting of the Society for Analytical Cytology, Breckenridge, CO, September 4-9 (1988).

McConnell, T. S., L. S. Cram, N. A. Baczek, J. J. Fawcett, M. L. Luedemann, and M. F. Bartholdi. Chromosome analysis by flow cytometry, Association of Cytogenetics Technicians Meeting, Kingston, Ontario, August 18-21 (1988).

Mudgett, J. S., G. F. Strniste, D. de Bruin, and M. A. MacInnes. Partial characterization of the putative human repair gene ERCC-5, Joint Meeting of the American Society for Cell Biology and the American Society for Biochemistry and Molecular Biology, *J. Cell Biol.* **107**, 520a (1988).

Nagasawa, H., D. J. Chen, J. B. Little, and G. F. Strniste. Study of radiation effects on G_1-phase, x-ray sensitive Chinese hamster (CHO) cell mutants, 36th Annual Meeting of the Radiation Research Society, **#Dk-8** (1988).

Nagasawa, H., J. B. Little, and G. F. Strniste. Low-dose rate effects on human skin fibroblasts, 37th Annual Meeting of the Radiation Research Society, in press.

Nagasawa, H., S. Carpenter, W. C. Inkret, J. B. Little, M. R. Raju, and G. F. Strniste. Relationship between cell survival and chromosomal damage after exposure to low doses of α-particles, 37th Annual Meeting of the Radiation Research Society, in press.

Nguyen, D. C., M. Alexander, R. A. Keller, J. H. Jett, and J. C. Martin. Laser-induced fluorescence of flowing samples as an approach to single molecule detection, University of Alberta, Edmonton, Canada, July 11 (1988).

Nickoloff, J. A., J. Singer, and F. Heffron. Double-strand break induced recombination in yeast, UCLA Symposium on Mechanisms and Consequences of DNA Damage Processing, Taos, NM, January 24-30 (1988).

O'Donnell, J. M. Tolerance to the behavioral effects of centrally active beta adrenergic agonist clenbuterol, *Soc. Neuroscience Abstr.* **14** (1988).

O'Donnell, J. M. The role of beta adrenergic receptor subtypes in the mediation of the behavioral effects of antidepressants, Collect of Neuropsychopharmacology (1988).

Okinaka, R. T., and G. F. Strniste. The nature of mutations induced by 2-acetylaminofluorene derivatives in CHO cells, 16th International Congress of Genetics, Toronto, Canada (1988).

Okinaka, R. T., and G. F. Strniste. Aromatic amine-DNA adducts: Studies on repair and consequences of misrepair of this type of DNA damage in mammalian cells, DOE Contractors Meeting on Interaction of Chemicals with Living Systems, Albuquerque, NM (1988).

Okinaka, R. T., and G. F. Strniste. The spectrum of hprt mutations induced by N-hydroxy-2-acetylaminofluorene in a DNA repair-deficient Chinese hamster cell line, Joint Meeting of the American Society for Cell Biology and the American Society for Biochemistry and Molecular Biology, *J. Cell Biol.* **107**, 83a (1988).

Okinaka, R. T., and G. F. Strniste. Mutations induced by N-acetoxy-2-acetylaminofluorene in DNA excision repair-deficient and repair-competent Chinese hamster cells, *J. Cell. Biochem.*, **Suppl. 12A**, 347 (1988).

Okinaka, R. T., M. A. MacInnes, and G. F. Strniste. Transformation of mammalian cells by pSV2gpt plasmids following modification with 2-aminofluorene derivatives: The role of DNA excision repair, 19th Annual Meeting of the Environmental Mutagenesis Society (1988).

Raju, M. R. Flow cytometry in biology and medicine, Workshop on Physical Techniques in Medical Sciences, Calcutta, India, December 12-15 (1988).

Raju, M. R. Glimpses of particle radiobiology, EORTC Heavy Particle Therapy Meeting, Essen, Germany, October 28-30 (1988).

Raju, M. R. Nuclear particles in cancer treatment: Brief review, Workshop on Dosimetry of Particles, Bariloche, Argentina, November 14-18 (1988).

Raju, M. R. Nuclear particles in cancer treatment: Brief review, Workshop on Physical Techniques in Medical Sciences, Calcutta, India, December 12-15 (1988).

Raju, M. R. Self-knowledge: Is it necessary in science? Workshop on Physical Techniques in Medical Sciences, Calcutta, India, December 12-15 (1988).

Raju, M. R., S. Carpenter, M. N. Cornforth, J. P. Freyer, M. E. Schillaci, R. T. Sebring, and M. E. Wilder. Dependence of ultrasoft x-ray RBE on cell thickness, 36th Annual Meeting of the Radiation Research Society, Philadelphia, PA, April 16-21 (1988).

Raju, M. R., S. Carpenter, W. C. Inkret, M. E Schillaci, K. Thompson, D. T. Goodhead. Cell inactivation by alpha-particles, 36th Annual Meeting of the Radiation Research Society, Philadelphia, PA, April 16-21 (1988).

Ray, F. A., D. S. Peabody, L. S. Cram, and P. M. Kraemer. Genetic hypervariability and selection of neoplastic variants as a mechanism of transformation for SV40 large T protein, Gordon Research Conference, Newport, RI, August 15-19 (1988).

Reynolds, R. J. Repair of closely opposed pyrimidine dimers in UV-irradiated Chinese hamster ovary cells, UCLA Symposium on Mechanisms and Consequences of DNA Damage Processing, Taos, NM, January 24-30 (1988).

Rodriguez, M. M., and J. W. Heyser. Effect of exogenous proline on the growth of *Distichlis spicata* suspension cultures and their metabolism of L-[1-^{13}C]-proline. 3rd Annual Symposium, Southwest Consortium on Plant Genetics and Water Resources, Las Cruces, NM, April 18-19 (1988).

Saito, S., H. A. Crissman, L. S. Cram, and J. D'Anna. Flow cytometric and biochemical analysis of dose-dependent effects of sodium butyrate on human endometrial adenocarcinoma cells, 13th International Meeting of the Society for Analytical Cytology, Breckenridge, CO, September 4-9 (1988).

Salzman, G. C., S. P. Pederson, R. J. Beckman, R. B. Krall, and C. C. Stewart. Cluster analysis for flow cytometry data, 13th International Meeting of the Society for Analytical Cytology, Breckenridge, CO, September 4-9 (1988).

Saunders, G. C., J. C. Martin, J. H. Jett, and A. Perkins. A flow cytometric competitive binding assay for determination of actinomycin-D concentrations, 13th International Meeting of the Society for Analytical Cytology, Breckenridge, CO, September 4-9 (1988).

Sillerud, L. O., N. H. Fink, and C. H. Han. ^{13}C NMR analysis of the complex carbohydrates of millet cell walls, ACS 9th Rocky Mountain Regional Meeting, Las Vegas, Nevada, March 27-30 (1988).

Sillerud, L. O., N. H. Fink, C. H. Han, W. Kirsch, D. Boheimer, P. Wheelan, and T. H. Koch. ^{13}C NMR Measurements of CA (II) binding parameters for aminomalonic acids and peptides containing aminomalonic acid, ACS 9th Rocky Mountain Regional Meeting, Las Vegas, Nevada, March 27-30 (1988).

Sillerud, L. O., R. H. Griffey, C. Fenoglio-Preiser, S. Shepard, and K. R. Halliday. *In vivo* ^{13}C NMR spectroscopy of the human prostate, *Proc. Soc. Magn. Reson. Med.* **7** 250 (1988).

Sillerud, L. O., C. F. Kirsch, G. Miller, R. Pennino, J. A. Jackson, and R. H. Griffey. *In vivo* proton 9.4 tesla nuclear magnetic resonance spectroscopy and 4.7 tesla imaging of rat sciatic nerve, *Clinical Research* **36** 165A (1988).

Sillerud, L. O., D. B. van Hulsteyn, and R. H. Griffey. NMR imaging of ^{13}C with proton sensitivity, 2nd European Congress of NMR in Medicine & Biology, Berlin, West Germany, June 21-25 (1988).

Sillerud, L. O., D. B. van Hulsteyn, and R. H. Griffey. NMR imaging of ^{13}C with proton sensitivity, *Proc. Soc. Magn. Reson. Med.* **7** 602 (1988).

Sinex, D. G., and L. P. McDonald. Auditory nerve fiber responses predict the discriminability of VOT syllables, *J. Acoust. Soc. Am.* **83**, Suppl. 1, S98 (1988).

Sinex, D. G., and L. P. McDonald. Discriminability of stop-consonant syllables related to auditory nerve fiber discharge patterns. Abstracts of the Eleventh Midwinter Meeting of the Association for Research in Otolaryngology (1988).

Sneed, R. A., A. P. Stevenson, and C. C. Stewart. A comparison of leukocyte infiltration into an immunogenic and a nonimmunogentic murine tumor, Society for Leukocyte Biology (RES Society) Meeting, Washington, DC, October 27-30 (1988).

Sneed, R. A., A. P. Stevenson, and C. C. Stewart. Characterization of the leukocytes infiltrating immunogenic and nonimmunogenic tumors in the BALB/C mouse, American Association for Laboratory Animal Science (AALAS), Detroit, MI, October 9-13 (1988).

Sneed, R. A., A. P. Stevenson, and C. C. Stewart. Characterization of the leukocytes infiltrating immunogenic and nonimmunogenic tumors in the BALB/C mouse, Laboratory Animal Section of 1988 American Veterinary Medical Association Annual Meeting and Scientific Program, Portland, OR, July 18-21 (1988).

Stallings, R. L., R. K. Moyzis, L. L. Deaven, J. L. Longmire, and C. E. Hildebrand. Physical mapping approaches for human chromosome 16: Progress and perspectives, Workshop on International Cooperation for the Human Genome Project, Valencia, Spain, October 24-26 (1988).

Stavert, D. M., and B. E. Lehnert. Concentration versus time is the more important exposure variable in nitrogen dioxide-induced acute lung injury, *The Toxicologist* **8**(1), p. 556A (1988).

Stavert, D. M., and B. E. Lehnert. Window of susceptibility for post-exposure exercise potentiation of nitrogen dioxide induced lung injury, Annual Meeting of the American Thoracic Society, Las Vegas, NV, May 8-11, *Am. Rev. Respir. Dis.* **137**, p. 354A (1988).

Steinkamp, J. A. Cell sorting, 3rd Course on Flow Cytometry Methods and Applications, Villejuif, France, June 6-11 (1988).

Steinkamp, J. A., J. C. Martin, B. F. Bentley, and H. A. Crissman. Phase-resolved measurement of fluorescence lifetimes, 13th International Meeting of the Society for Analytical Cytology, Breckenridge, CO, September 4-9 (1988).

Stevenson, A. P., R. C. Habbersett, and C. C. Stewart. Identification of human leukocyte subsets by multivariate flow cytometry analysis, 13th International Meeting of the Society for Analytical Cytology, Breckenridge, CO, September 4-9 (1988).

Stevenson, A. P., R. C. Habbersett, and C. C. Stewart. The radiation sensitivity of resting and of PHA-stimulated human leukocytes subsets from young and aging donors, 36th Annual Meeting of the Radiation Research Society, Philadelphia, PA, April 16-21 (1988).

Stewart, C. C., R. C. Habbersett, S. J. Stewart, R. A. Sneed, A. P. Stevenson, C. Willman, J. Bender, J. Houch, and D. Wirt. Multiparameter flow cytometry, 13th International Meeting of the Society for Analytical Cytology, Breckenridge, CO, September 4-9 (1988).

Strniste, G. F., D. J. Chen, L. S. McCoy, J. W. Nickols, R. T. Okinaka, and J. G. Tesmer. Restoration of rodent cell radiation resistance by human repair genes, *J. Cell Biochem.* **Suppl. 12A,** 245 (1988).

Tomei, F. A., L. R. Barton, C. Lemanski, R. J. Sebring, and L. O. Sillerud. Adaptation of cultures of *Desulfovibrio desulfuricans* and *Wolinella succinogenes* to growth in the presence of selenate and selenite, American Society for Microbiology, Las Cruces, NM, October 14 (1988).

Van Dilla, M. A., and L. L. Deaven. The National Laboratory Gene Library Project, Hanford Life Sciences, Richland, WA, October 18-20 (1988).

van Hulsteyn, D. B., R. H. Griffey, and L. O. Sillerud. Generalized chemical shift imaging using gradient refocusing for phase encoding the chemical shift information, *Proc. Soc. Magn. Reson. Med.* **7,** 1053 (1988).

Voelz, G. L., A. P. Stevenson, and C. C. Stewart. Lymphocyte studies on plutonium workers, Versailles Workshop on Biological Assessment of Occupational Exposure to Actinides, Versailles, France, May 30 - June 2 (1988).

Zhao, Z., J. W. Heyser, and H. J. Bohnert. Gene expression in *Distichlis spicata* cells in culture during adaptation to high salt, 3rd Annual Symposium, Southwest Consortium on Plant Genetics and Water Resources, Las Cruces, NM, April 18-19 (1988).

Zhao, Z., J. W. Heyser, and H. J. Bohnert. Isolation and characterization of putative salt tolerance genes from *Distichlis spicata*, *Plant Physiol.* **83(4)Suppl.** p. 151, (1988).

AWARDS

Bulcan, T. N. 1988 R&D 100 Award, Optical Microrobot Single-Cell Manipulator and Analysis System.

Deaven, L. Distinguished Performance Award, Los Alamos National Laboratory.

Rodriguez, M. Santa Fe High School student. Won the following honors for his research on the metabolism of exogenous proline by saltgrass cells which was performed in the laboratory of J. Heyser:

- First place, Botany, New Mexico Science and Engineering Fair, Socorro, NM, April 1988.
- Fourth place, Botany, International Science and Engineering Fair, Knoxville, TN, May 1988.
- 1988 Honors Group, Westinghouse Science Scholarships and Awards, Science Talent Search

Valdez, J. Distinguished Performance Award, Los Alamos National Laboratory.

PATENTS AWARDED

Jackson, P. J., E. Delhaize, N. J. Robinson, C. J. Unkefer, and C. Furlong Metal-Binding Polypeptides, U.S. Patent (filed).

Johnston, R. Interferometic Apparatus and Method for Detection and Characterization of Particles Using Light Scattered Therefrom, U.S. Patent, 1988 #4764013.

Johnston, R. Apparatus and Method for Performing Two-Frequency Interferometry, U.S. Patent (filed).

Longmire, J. L., A. K. Lewis, and C. E. Hildebrand. Nucleic Acid Isolation, U.S. Patent (filed).

Ray, A., L. S. Cram, and W. R. Galey. Electromicroinjection of Particles into Living Cells, U.S. Patent #4784737.

* U.S. GOVERNMENT PRINTING OFFICE: 1989—673-104/80084