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LAWRENCE BERKELEY LABORATORY
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# LIFE SCIENCES

Lawrence Berkeley Laboratory
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Lawrence Berkeley Laboratory is a multiprogram national laboratory managed by the University of California for the U.S. Department of Energy (DOE). The Laboratory has 3,000 employees, and its budget of over $223 million supports a wide range of unclassified research activities in fields ranging from astrophysics to human genetics.

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# FOREWORD

LIFE SCIENCES RESEARCH HAS A LONG HISTORY at LBL. Laboratory founder Ernest O. Lawrence invented the first modern particle accelerator, the cyclotron, in 1930. Within 10 years, he and his brother, John Lawrence, a physician, started using the cyclotron to treat cancer patients. The tradition of nuclear medicine thus begun continues today in LBL's Research Medicine and Radiation Biophysics Division. LBL's Chemical Biodynamics Division also has a long and distinguished history: The Division is the outgrowth of a laboratory established in 1945 to conduct basic research on the dynamics of living cells and the interaction of radiant energy with organic matter. Research in Chemical Biodynamics has included Melvin Calvin's Nobel prize-winning elucidation of the biochemical pathway of photosynthesis.

Through the 1970s, however, life sciences research remained a secondary endeavor at LBL, and the Lab remained known primarily as an international leader in high-energy and nuclear physics. Today, fulfilling a vision that began more than 10 years ago, LBL has emerged as a true multiprogram laboratory, where the life sciences are playing an ever-increasing role. In 1988, the Cell and Molecular Biology Division was established to further basic research in modern biology: The Division supports programs in gene expression, molecular genetics, and the basis of radiation-induced cellular damage.

The multidisciplinary spirit that has emerged at the Laboratory is well illustrated by two major life sciences initiatives: the LBL Human Genome Center and the Life Sciences Center at LBL's Advanced Light Source. The Human Genome Center is part of a growing international effort to map, and eventually to sequence, the human genome. The Center's achievements will represent the efforts of not only the Cell and Molecular Biology and Chemical Biodynamics Divisions, but also Engineering, Materials Sciences, and Information and Computing Sciences. The Life Sciences Center to be established at LBL's Advanced Light Source will fulfill the needs of biomedical researchers for full experimental access to new synchrotron radiation facilities. In addition to beamlines and experimental end stations, the Life Sciences Center will provide supporting laboratories and office space for scientists from across the U.S. Like the Genome Center, the Life Sciences Center involves individuals from a variety of disciplines: To make the Life Sciences Center a reality, researchers from all three life sciences divisions are working with engineers and physicists associated with the Advanced Light Source project.

We look forward to continued collaboration among the life sciences divisions—and between these divisions and the rest of the Laboratory—to ensure LBL's position at the forefront of biological and medical research through the 1990s and beyond.

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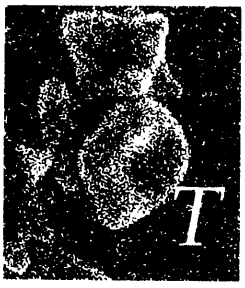
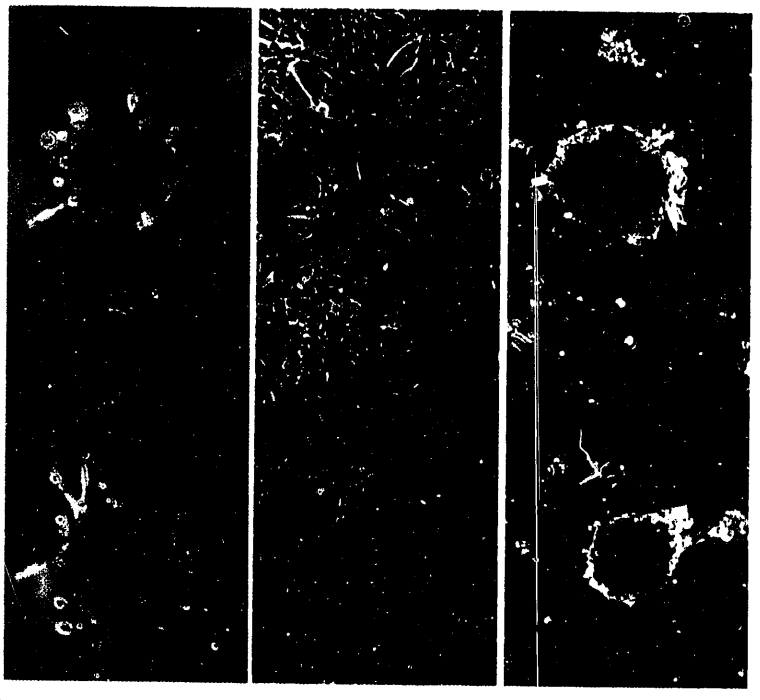


# CELL AND MOLECULAR BIOLOGY

THE CELL AND MOLECULAR BIOLOGY DIVISION pursues multidisciplinary basic research in the broad areas of gene expression and molecular genetics. Its activities range from studies of macromolecular structure by electron crystallography to research on mechanisms of carcinogenesis (cancer development) and use of LBL's Bevalac accelerator to study basic radiation biology. In addition, the harmful and beneficial effects of many different types of radiation—ranging from ion beams produced by the Bevalac to ultraviolet radiation from the sun—are being studied at the chemical, molecular, cellular, tissue, and organ levels of biological organization.

*The development of a deeper understanding of gene expression is a unifying research goal in the Cell and Molecular Biology Division.*

The development of a deeper understanding of gene expression is a unifying research goal: Programs are united by their ultimate focus on understanding how an organism's genetic program is expressed and regulated, how it maintains itself, how it is affected by radiation and toxic substances, and how it goes wrong in tumor formation. This includes elucidating the biological effects of DNA-damaging agents and establishing both the health risks associated with DNA damage and the genetic basis of protection against such damage. Much of the work centers around the nature of carcinogenic transformation and the molecular mechanisms of DNA repair.



*Mouse mammary epithelial cells grown on reconstituted basement membrane (left and right) form structures resembling those of mammary glands during pregnancy and lactation. In contrast, cells grown on plastic (center) are randomly oriented.* *See "Cell-Cell and Cell-Matrix Effects," page 10.*

The Cell and Molecular Biology Division is also the administrative home of the Human Genome Center, one of three centers designated by the Department of Energy (DOE) for research aimed at ultimately mapping and sequencing the human genome—all the genes that determine the structure and function of the human body. In line with the DOE's strategy for developing advanced tools for the effort before undertaking large-scale mapping or sequencing projects, the Center is the focus of activities in several LBL divisions, including Engineering, Materials Sciences, and Information and Computing Sciences, as well as the three life sciences divisions. The connection to Cell and Molecular Biology is a natural one: Once the human genome project bears its ultimate fruit, the real work can begin, namely, deciphering in detail the intricate puzzle of gene expression.

## RADIATION BIOLOGY

*Studies in radiation biology serve as a firm link to the history of life sciences research at LBL, as well as a bridge to the radiotherapy and radiosurgery programs in the Research Medicine and Radiation Biophysics Division (pages 46–48). At the same time, they are closely tied to our studies of carcinogenesis and repair of DNA—the genetic material of living organisms. As it has for a decade and a half, much of the interest at LBL concerns the effects of heavy ions (charged particles), which, compared with x-rays, deposit large amounts of energy over very short distances at the end of their paths. Investigations of the interactions of heavy ions with normal tissue aim at defining tolerance limits and the risks of carcinogenesis associated with exposure to these particles. Studies with tumor tissue are directed toward determining optimal strategies for tumor treatment, including dose, particle type, and treatment interval. Finally, fundamental radiobiological research is focused on understanding and characterizing biological processes such as DNA damage and repair, as well as physical phenomena such as the fragmentation of ions into particles of lower charge and mass.*

### Radiological Physics

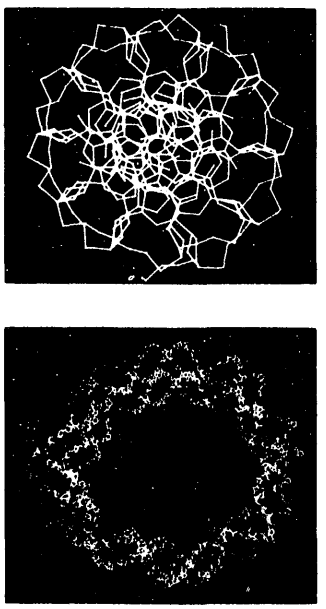
We have developed a theoretical model that assesses DNA damage from ion beams and other forms of ionizing radiation—that is, radiation that can dissociate molecules to produce either charged particles or highly reactive "free radicals" that can attack and damage DNA and other important compounds in living cells. One way that ionizing radiation produces DNA damage starts with the splitting of a water molecule to produce a hydrogen atom and a hydroxyl radical. Attack by a hydroxyl radical is called an "indirect mechanism" of DNA damage. Alternatively, the radiation can directly strike and damage a DNA molecule. Our model shows that most of the DNA damage from energetic electrons and other forms of low-LET radiation is via the indirect mechanism. In contrast, high-LET radiation, such as a beam of neon ions, produces DNA breaks primarily by the direct effect. (Low-LET and high-LET radiation are defined, respectively, by a low or high rate of energy transfer as the radiation travels through a medium.) This may have significant implications for radiotherapy, because indirect damage can be prevented by drugs that scavenge, or elimi-

*Bill Holley (left) and Aloke Chatterjee have developed a theoretical model that assesses DNA damage by ionizing radiation. On the computer screen is an image of a hydroxyl radical attacking the sugar backbone of a DNA molecule, thereby inducing damage.*

nate, hydroxyl radicals. In addition, certain drugs can reverse damage by the indirect mechanism before it becomes permanent.

In living cells, DNA molecules consist of two strands of DNA bound together to form a double helix. A break in just one strand is usually rejoined quickly by repair enzymes. A double-strand break is much more difficult for the cell to repair and is therefore more likely to be a precursor of mutation, cancer, or cell death. Our computer model predicts the yields of both single- and double-strand breaks in DNA in its simple linear double-helix form. We are now extending the model to study energy deposition in higher-order DNA structures and the mechanisms of activation of cancer-causing oncogenes.

In a separate but related effort, we use statistical methods to simulate the patterns of ion formation caused by the slowing down of charged particles in liquid water and relate the size of ion clusters to "irreparable" and "repairable" damage to DNA. These ideas are incorporated into our theory of lethal and potentially lethal lesions. This theory, which presently applies only to cell killing, suggests (1) relationships between repair of sublethal and potentially lethal damage and (2) reasons why various normal tissues differ in their responses to the effects of radiation.



*The DNA of all plants and animals forms loops around structures called nucleosomes, which consist of proteins. The top figure shows a computer-generated image of the DNA loops, but not the proteins. The bottom figure shows a computer-generated end-on view of DNA spiraling around nucleosomes. LBL researchers have found that nucleosomes tend to protect DNA from damage.*

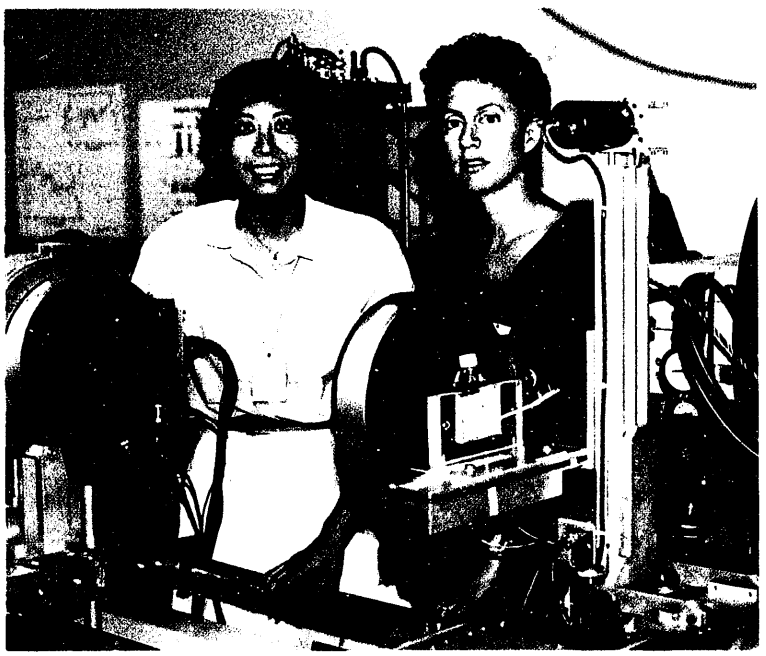
### Radiation-Induced DNA Damage and Repair

In seeking to understand the effects of ionizing radiation on genetic material, we have focused much of our effort in three areas: detection of radiation-induced DNA damage and repair, investigation of the LET-dependence of DNA breaks and rejoining, and evaluation of neon-ion–induced rearrangements in a single human chromosome. We hope to uncover the mechanistic basis for the well-established observation that the effectiveness of radiation in killing cells depends greatly upon its LET value. If we postulate that unrepaired or misrepaired DNA breaks are the only types of damage that influence cell survival, we can perhaps unravel the LET dependence of cell killing by looking separately at the damage and repair processes. This is made possible by a technique called premature chromosome condensation, which allows us to halt cellular processes and examine DNA breaks in individual cells. The results show that both the time course and the extent of DNA-break rejoining depend on the LET of the radiation. We plan to extend these studies to other types of cell damage, including mutations (heritable changes in DNA).

### Radiation-Induced Cell Mutation and Carcinogenic Transformation

Apart from cell death, genetic damage caused by ionizing radiation can have a second and more insidious effect: cell mutation and cancerous proliferation. Our approach to studying this process has several facets. One project focuses on the differences between radiation-induced mutations at two human genes, TK and HPRT. Is the spectrum of DNA structural alterations seen in radiation-induced mutants dependent upon the density of energy deposition? Will the placement of genes at different chromosomal locations have an influence on the types of mutants recovered?

In recent work using TK6 lymphoblasts (a human male-derived cell line), we have found a dramatic gene specificity for the effects of x-rays, as well as accelerated heavy ions produced by the Laboratory's Bevalac accelerator. For all types of radiation studied to date, mutations are produced more readily in the TK gene than in the larger HPRT gene. This surprising finding may be associated with the chromosomal context in which these two genes are located: TK is located on chromosome 17, two copies of which are normally present in human cells, whereas HPRT is located on the single X chromosome. It is possible that HPRT is linked along the chromosome to genes essential to cell survival, which means that extensive radiation damage—such as that associated with heavy ions—would be more likely to cause cell death than a heritable mutation. In addition, the mechanisms used to process initial radiation damage to the final product, namely heritable alterations in DNA, may be more numerous when two copies of the gene are present.



*Research associate Polly Chang (left) and Amy Kronenberg prepare to irradiate TK6 cells in a mutation experiment in the biology beam line at the Bevalac. They have found a dramatic gene specificity for the effects of x-rays and accelerated heavy ions.*

Pursuit of this work, directed especially toward an explanation for the observed gene specificity, promises a deeper understanding of the mechanisms of cell mutation and carcinogenesis induced by ionizing radiation.

A second effort centers around some of the events that accompany carcinogenic transformation, including chromosomal alterations in the vicinity of known proto-oncogenes (genes that can become cancer-causing oncogenes) and changes in gene expression, as measured by changes in RNA levels. An early result is the finding that cell transformation occurs when ionizing radiation causes two DNA strand breaks as a result of energy deposition or attack by radicals at DNA sites within 80 Å of one another (one Å equals one ten-billionth of a meter).

We are also looking at tumor development in several systems, including the Harderian gland, which is found in most vertebrates (although not in human beings) and functions in lubrication of the globe of the eye. Using the mouse Harderian gland, we are examining the long-term biological response of normal tissue to high-LET radiation. This work will contribute to the determination of the carcinogenic risk associated with high-LET radiation at low doses and will be particularly valuable in understanding the risk from environmental radiation such as plutonium alpha particles, fission neutrons, and high-energy particles from outer space.

Our strategy is to compare the tumor-induction effectiveness of cobalt-60 gamma rays (a form of low-LET radiation) to that of several high-LET particle beams from the Bevalac: helium, carbon, neon, argon, iron, and niobium. We have demonstrated that, unlike cobalt-60 gamma rays, high-LET radiation causes an increase in the number of Harderian tumors even at very low doses.

Using the Harderian gland as a model system, we have gone far in establishing the relationship between energy deposition and cancer induction. In future experiments, we will study the relationship between cancer risk and effects per particle by looking at tumor induction as a function of particle traversal per cell nucleus.

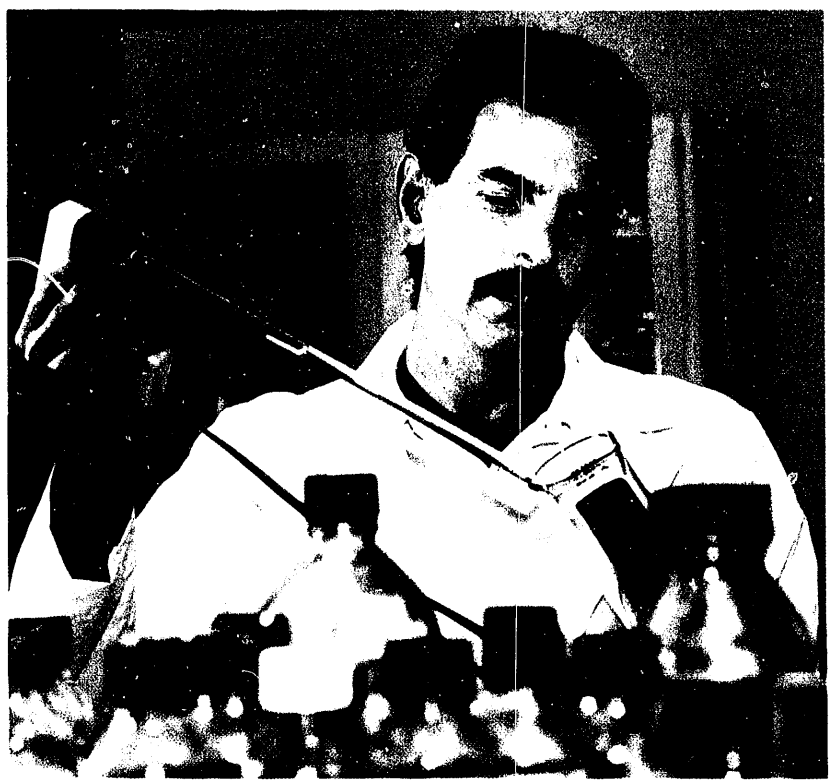
## CHEMICAL- AND UV-INDUCED DNA DAMAGE AND REPAIR

*The release of a normal mammalian cell from the restraint of differentiation, controlled cell division, and a finite life span produces a cancer cell. The succession of steps that produces this transformation from a normal to a cancerous cell is not well understood, but research efforts in many areas of cell and molecular biology, including the studies in radiation biology outlined above, suggest a requirement for repair of DNA damage if carcinogenesis is to be prevented. Unrepaired point mutations, for example, are among several mechanisms apparently capable of "turning on" oncogenes, and individuals with genetic-repair-deficiency diseases are extremely cancer prone. Our activities in the area of DNA damage and repair include cloning of human DNA repair genes by complementation of the defect in human repair-deficient cells, research on how structural constraints and organization of the cell's genome affect repair processes, studies of inducible responses to DNA damage, and cell culture studies of replication of damaged DNA templates.*

### RNA Polymerase and DNA Repair

Previous work in the Cell and Molecular Biology Division established that the efficiency of DNA repair varies according to whether or not the damaged gene is actively being transcribed into RNA—a prerequisite step to protein synthesis. Repair of damage induced by either ultraviolet (UV) light or aflatoxin B1 (a mold toxin) was found to be more rapid in three transcribed human genes than in the genome overall. Repair rates for UV-induced damage in a nontranscribed gene and in a pseudogene (which is never transcribed) were about the same as the average rate for the entire genome; the rates for repair of aflatoxin-induced damage in these genes were lower than the average rate. Inducing higher levels of transcription increased repair efficiency in the transcribed genes but had no effect on the nontranscribed ones. Thus, repair efficiency appears to depend on both the type of damage introduced into a DNA sequence and the transcriptional state of the gene.

*Tony Leadon has shown that repair of DNA damage in the transcribed strand of a gene is more efficient than repair in the nontranscribed strand.*



In more recent work, we have extended these findings by measuring repair in the transcribed and nontranscribed DNA strands of genes. We have found a significant (threefold) increase in the efficiency of repair in the transcribed strand, compared with the nontranscribed strand. Repair on the nontranscribed strand of an active gene, on both strands of an inactive gene, and on both strands of an active gene's regulatory region was comparable to that of the genome overall. Also, inducing higher levels of transcription selectively increased repair efficiency only on the transcribed strand. When cell cultures were treated with an inhibitor of the enzyme that catalyzes RNA synthesis, preferential repair on the transcribed strand was eliminated, and conditions that had previously induced transcription were found to have no effect on repair efficiency. Taken together, these recent observations suggest that initiation of repair on the transcribed strand of a gene is independent of repair on the nontranscribed strand and that repair may directly involve the transcriptional complex—the multiprotein assembly that carries out transcription.

Ongoing work is aimed at further elucidating the mechanisms responsible for this preferential repair. Possibilities include the association of repair enzymes with the transcriptional complex. Alternatively, arrest of transcription at the site of DNA damage might serve as a repair-initiating signal. The results of our work will be important not only for an understanding of the mechanism and biological consequences of preferential repair in active genes, but also because unrepaired damage in "silent" but potentially active genes, including proto-oncogenes, has significant implications for human health.

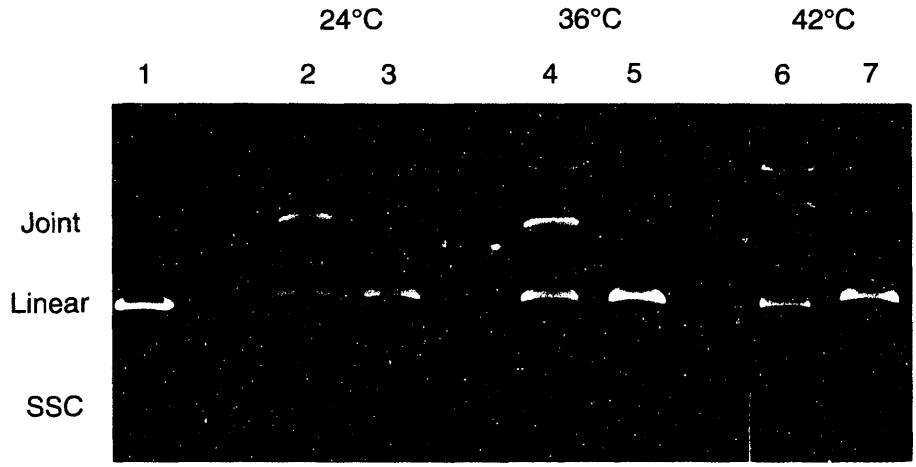
## YEAST STUDIES

*Significant effort centers around research with yeast: We are studying recombination (exchange of genetic material between chromosomes) in* Saccharomyces cerevisiae, *both during cell division and as a DNA repair mechanism, and we are using yeast to clone human genes. We also maintain a unique yeast stock center as an international resource, and we regularly update our widely used compilation of yeast genetic mapping data.*

### Protein Essential for Genetic Repair

Recombination is a significant means by which potentially oncogenic chromosomal damage is repaired: One chromosome, in effect, uses its homolog (a chromosome containing the same genes as another) as a template. In addition, a specialized form of recombination is required for the normal development of the immune system. The goals of recombination studies in model systems are to characterize the molecular mechanisms of chromosomal recombination and to identify the major recombination genes, together with their protein products. If sufficient similarity can be shown to exist between human cells and model systems such as yeast, the models can be used to extend our understanding of vital DNA repair processes in humans.

In an effort to illuminate this critical process of recombination, we have isolated and characterized three temperature-sensitive, recombination-deficient mutants of the yeast *Saccharomyces cerevisiae*. Each embodies a deficiency in a single recombination gene: REC1, REC3, or REC4. Of particular interest, the REC1 gene encodes a protein essential for DNA strand transfer between homologous DNA molecules—the key step in chromosomal recombination. We have demonstrated that the thermosensitive version of this protein is active at temperatures that would permit genetic recombination in a living organism, and inactive at temperatures that would inhibit genetic recombination.

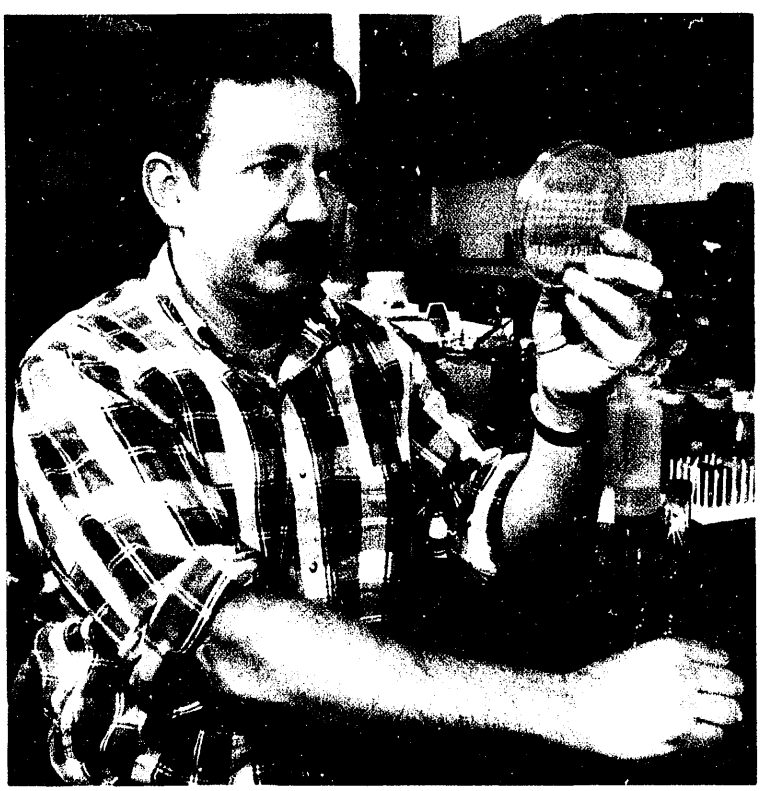



*The* REC1 *DNA strand-transfer protein is required in yeast for recombination and for repair of x-ray-induced DNA damage. The row labeled "joint" shows recombinant DNA, i.e., DNA in which strand transfer has occurred. In lane 1, no strand-transfer protein has been added. Lanes 2, 4 and 6 show strand transfer by the normal* REC1 *protein. Lanes 3, 5, and 7 show that a mutant form of this protein is active at 24°C and 36°C, but inactive at 42°C. The mutant protein is thermosensitive in living cells, as well as in the extracts used in this type of experiment. (SSC = single-stranded circular viral DNA. Linear = double-stranded linear DNA.)*

Human cells contain an analogous DNA strand-transfer protein, and studies are in progress to determine whether a plasmid (a circular piece of DNA used to introduce foreign genes into a host cell) containing this analog can restore recombination competence to mutant yeast. Similarity between the human and yeast systems would open the door to studies that might better define the process of chromosomal repair in human cells.

### Cloning of Multifunctional Human Genes in Yeast Cells

We have cloned three multifunctional human genes in yeast cells by using human genes to compensate for mutations in the corresponding yeast genes. The human genes came from a cDNA library constructed by collaborators at Chiron Corporation in Emeryville, California. (cDNA, used in cloning, contains only DNA segments that actually code for proteins.) This is the first time a human cDNA library has been constructed in a yeast plasmid.



*CMB researcher David Schild examines a yeast culture. In yeast cells, he has cloned three multifunctional human genes, one of which may be associated with Down's syndrome.*

All three genes are involved in synthesis of the purine DNA bases, adenine and guanine. One of these genes, the GART gene, is particularly important because it is located in the region of chromosome 21 associated with Down's syndrome and may even be responsible for some of the symptoms of this disease. Genes involved in purine synthesis are also of medical interest because chemotherapeutic drugs used to treat cancer block synthesis of the DNA bases. Cloning of the purine synthesis genes may therefore lead to a better understanding of how chemotherapeutic agents work.

In evolution, there has been a tendency toward combining the functions of purine-synthesis genes. In bacteria, each of the three "GART" steps in purine synthesis is carried out by a different enzyme that has its own gene. Yeast cells, in contrast, have two enzymes for the GART functions—one bifunctional and one unifunctional—and two corresponding genes; human cells, which have just one GART gene, have one GART protein with three different enzymatic activities. Cloning and sequencing of multifunctional human genes may aid in the study of protein evolution.

## DIFFERENTIATION AND CARCINOGENESIS

*One of the deepest mysteries of gene expression is how cells with identical genetic programs differentiate during the maturation of complex organisms to become, for example, such diverse tissues as muscle, nerve and the epithelium that covers body surfaces and lines organs. Accordingly, the generation of tissue specificity—and its loss during malignancy—are among the pivotal research themes in modern biology. In our own research in this broad area, we are taking several approaches. One of the most important has been to culture human and rodent mammary epithelial cells as systems for the study of normal growth, differentiation, and carcinogenic transformation. In particular, we are interested in the role of cell-cell and cell-matrix interactions in gene expression and differentiation in these systems. Since the development of mammary glands is subject to hormonal regulation, we are also interested in determining the relative importance of hormones versus cell-cell and cell-matrix interactions, as well as how the interplay among hormones and various cellular factors dictates mammary gene expression. Another approach to the same broad issue of differentiation and carcinogenesis is to use avian virus systems to study critical features of oncogene expression and the influence of environmental factors. And finally, we are intensively studying the process of hematopoiesis—the differentiation of blood cells from a multipotential stem cell—as a model for the processes that take place during embryogenesis.*

### Biology of Human Mammary Epithelial Cells

The central goal—and primary achievement—of our research on human mammary epithelial cells has been to establish a well-defined culture system that can be used for widespread investigations of epithelial cell development. Indeed, we have established a normal cell population which is capable of long-term growth but has the characteristics of a stem cell population: Its characteristics can be modulated in culture by age, medium components, and the nature of the surface to which the cells are attached. With this common resource now available, significant new findings are emerging from a number of laboratories, including insights into the normal mechanisms that control cell proliferation and differentiation, as well as how these normal processes are altered in immortal cell lines and in the process of malignant transformation.

In our own labs during the past year, work has focused on the role of growth factors and proto-oncogenes in cell proliferation and on the interaction of epithelial cells with the carcinogen benzo[a]pyrene (BaP). We previously showed that human mammary epithelial cells readily convert BaP to its ultimate carcinogenic form, BPDE, whereas mammary fibroblasts (cells that give rise to connective tissue) are much less capable of this reaction. More recently, we identified a previously uncharacterized BaP metabolite

in mammary epithelial cells and found that it is an extremely potent and selective inhibitor of the key step in the generation of BPDE. It thus appears that the activation of BaP to its carcinogenic form in these cells is self-limited through a novel feedback inhibition mechanism.

### Cell-Cell and Cell-Matrix Effects

Almost all organs are composed of at least two tissue components: epithelial parenchyma (the essential and distinctive tissue of the organ) and mesenchymal stroma (the supporting framework of connective tissue). Interaction between these two components is crucial to proper development, to the formation of tissues and organs, and to maintenance of the differentiated state. Any disturbance to this interaction can lead to uncontrolled growth, aberrant tissue behavior, and carcinogenesis.

Using mammary gland cells from the mouse, we have explored the role in cell differentiation of the extracellular matrix (ECM), which consists of material secreted by both epithelial and stromal cells. Among our findings, we showed that the ECM shares with hormones the responsibility for regulating tissue formation and gene expression. We also demonstrated a dramatic effect of the ECM on the architecture of epithelial cells in culture: On reconstituted basement membranes (a type of ECM), separated cells will form alveoluslike structures remarkably similar to mammary glands during pregnancy and lactation. Our latest findings indicate possible response elements to ECM in the promoter regions (the sites where transcription of the genetic message begins) of the genes for milk proteins. In the future, we will explore the nature of these gene sequences and how ECM ultimately regulates gene expression in normal and malignant cells.

### Hormonal Effects

The female steroid sex hormones, estrogen and progesterone, are important for the development and differentiation of normal mammary epithelial cells. It has been known for more than a century that these hormones are also involved in the growth of human mammary carcinomas. Many of the effects of steroid hormones in their target tissues require the action of intracellullar steroid-specific receptor proteins that modulate the expression of a network of genes whose actions culminate in the tissue's characteristic response to the hormones.

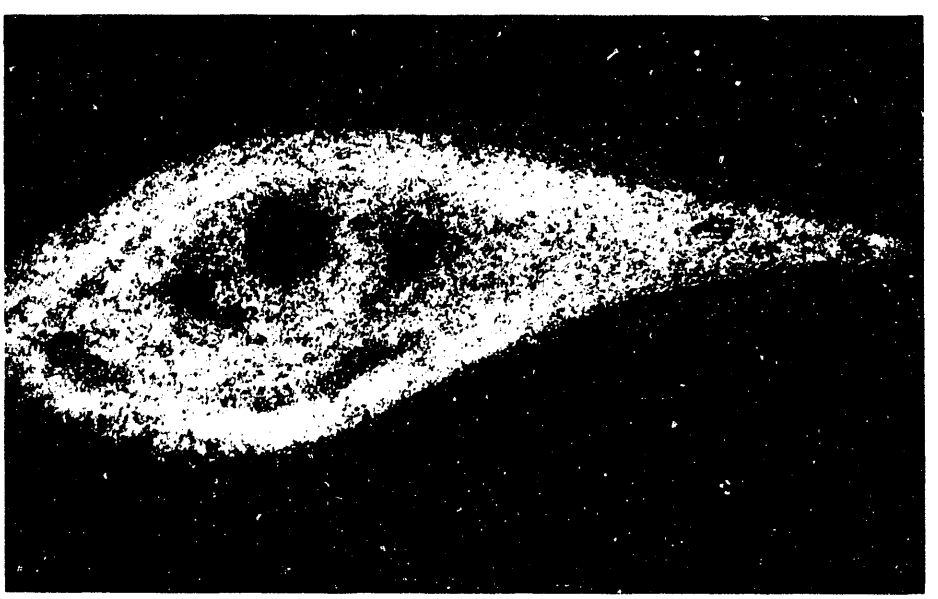
Alterations in the synthesis and function of estrogen and progesterone receptors can cause disruption of the mechanisms responsible for maintaining a stable state of mammary epithelial cell proliferation. This can ultimately lead to cancer. Using the mouse mammary gland as a model system, we are therefore seeking to identify the fundamental processes underlying the synthesis and function of estrogen and progesterone receptors. An important finding to emerge from our studies is that, while normal mammary development and differentiation are under the regulation of estrogen and progesterone, the actions of these hormones are themselves subject to modulation by the developmental state of the mammary epithelial cell. For example, the estrogen-regulated synthesis of progesterone recep-

tors is impeded during terminal differentiation of mammary glands. Thus, it appears that cell-specific factors associated with terminal differentiation may alter the ability of estrogen receptors to trigger expression of the progesterone receptor gene. To determine the identity of these factors and to facilitate the study of how estrogen and a cell's developmental stage affect expression of the progesterone receptor gene, we have recently cloned, sequenced and expressed the cDNA that encodes the mouse progesterone receptor.

### Viral Carcinogenesis

Our knowledge of the genetics and molecular structure of RNA tumor viruses is not matched by our understanding of viral carcinogenesis. Whereas cell lines and tissue culture models have been very fruitful in addressing aspects of viral transformation, the ultimate answer to how viruses cause cancer must come from studies in living organisms. We are therefore using a replication-defective avian Rous sarcoma virus (which carries an oncogene) to study expression and inhibition of tumor formation in chick embryos. One dramatic result is the finding that the embryonic environment of the chick suppresses the oncogenic potential of the viral gene. After four-day-old chick embryos are microinjected with the virus, we readily detect evidence of viral integration and replication, as well as expression of the active oncogene; nonetheless, embryonic development proceeds normally. In the embryos, only vascular endothelial cells are susceptible to the action of the oncogene. We are now investigating the reasons for this susceptibility, as well as the reasons why other tissues are refractory.

In related studies with newborn chicks, we have also found that wounding plays a role as a cocarcinogen in tumor formation following injection with the Rous sarcoma virus. Chick hatchlings infected with this virus



*Cross-section through a chicken tumor induced by the wound-associated protein TGF-β (transforming growth factor beta) in the presence of Rous sarcoma virus. The white grains (radiotracer probes) in the micrograph indicate the widespread presence of Rous sarcoma virus mRNA.*

*CMB Division Director Mina Bissell and Michael Sieweke have shown that TGF-β is a central mediator in the tumor-promoting effects of wounding.*

rapidly form tumors at the site of experimental wounds. We found that a small protein, transforming growth factor beta (TGF-β), known to be involved in growth regulation in many tissues, was produced at the wounds before tumor formation. Further, when a pure preparation of this molecule was administered to virus-infected chickens, it effectively mimicked wounding and produced tumors at the site of application. Other growth factors either are ineffective or need to be administered at much higher levels. Thus TGF-β appears to be a central mediator in the tumor-promoting effects of wounding.

Besides shedding light on a long-standing problem, this finding has important implications for our understanding of tumor progression in general. Levels of active growth factors such as TGF-β in a given tissue may well determine whether an initiated tumor cell can develop its full cancerous potential.

**Gene Expression in Normal and Cancerous Cell Lines**

Identification of gene products—that is, proteins—that play a central role in the progression of epithelial cancer is an important initial step in deciphering the biochemistry of the disease. In an effort to understand better the processes whereby normal mammary epithelial cells become malignant, we have begun a search for specific genes whose expression might be relevant to these processes. Such genes would include those directly altered by a carcinogenic event, those involved in carcinogen-altered metabolic pathways, and those that normally present obstacles to tumor development.

One method of identifying such gene products is by comparison of cells from normal tissue with those from precancerous or cancerous tissue. The approach has been to identify genes that are not actively being transcribed into mRNA (messenger RNA). This is done by hybridizing single-stranded cDNA from one cell type with an excess of mRNA from a second cell type, then looking for cDNA that remains unpaired. Since each mRNA molecule directs the synthesis of a specific protein, unpaired cDNA must code for proteins that are not currently being produced in the second cell type.

So far, we have identified three proteins present in normal mammary epithelial cells but largely absent in malignant cells lines: (1) fibronectin, (2) keratin, and (3) vimentin. We have also found in normal cells an mRNA molecule, designated NB-1, whose protein product has not been characterized. NB-1 production decreased more than 50-fold in mammary cells after

carcinogenic transformation in culture. The cDNA encoding NB-1 bore no similarity to genes of known sequence.

### Tumor-Cell Detection Using Monoclonal Antibodies

Efforts are directed toward characterizing cell-membrane glycoproteins (proteins with carbohydrate components) of breast epithelial cells and breast tumor cells. One of the techniques that we are using is that of generating monoclonal antibodies (which recognize and bind to just one type of target molecule) against cell membranes. These antibodies are useful for basic studies of membrane biochemistry but also may have value as diagnostic tools for the detection of tumor cells. Additionally, some surface proteins of breast tumor cells are shed from tumors and appear in the blood serum of breast cancer patients. Monoclonal antibodies can be used to detect these proteins and consequently to predict the existence of latent tumors or monitor the success of surgical efforts to remove tumors.

Over the past few years we have generated several monoclonal antibodies that react with human breast tumor cells and have used them to study various aspects of tumor biology. We have also entered into a collaborative project with scientists at Triton Biosciences of Alameda, California, to examine the value of these antibodies as tools to monitor tumor-derived glycoproteins in the serum of breast cancer patients. Screening of approximately 50 cancer patients demonstrated that the monoclonal antibodies did indeed detect shed tumor glycoproteins in the serum. Ongoing experiments are directed toward determining the sensitivity of these antibodies and assessing whether they offer advantages over monoclonal antibodies that have been generated at other laboratories.

### Carcinogenic Potency

For several years we have been developing the Carcinogenic Potency Database, a standardized database of the published results of animal carcinogenesis tests. The database currently includes results of about 4,000 experiments on 1,000 different chemicals. To describe carcinogenic potency in an animal experiment, we estimate $TD_{50}$—the chronic dose rate (in milligrams per kilogram of body weight per day) that would induce tumors in 50 percent of test animals by the end of a standard lifetime. We have found that $TD_{50}$ values of rodent carcinogens vary more than 10 millionfold.

About half of the chemicals tested are carcinogenic in rats, mice, or both. Of 392 chemicals tested in both species, 76 percent of the rat carcinogens are positive in mice, and 70 percent of the mouse carcinogens are positive in rats. Prediction of carcino-genicity in one species based on that in the other is more reliable for mutagens than for nonmutagens and for substances that are toxic at low, as well as high, doses. An analysis of the predictive value of carcinogenicity at the 10 most common tumor sites indicates that most sites are good predictors of carcinogenicity at some site in the other species.

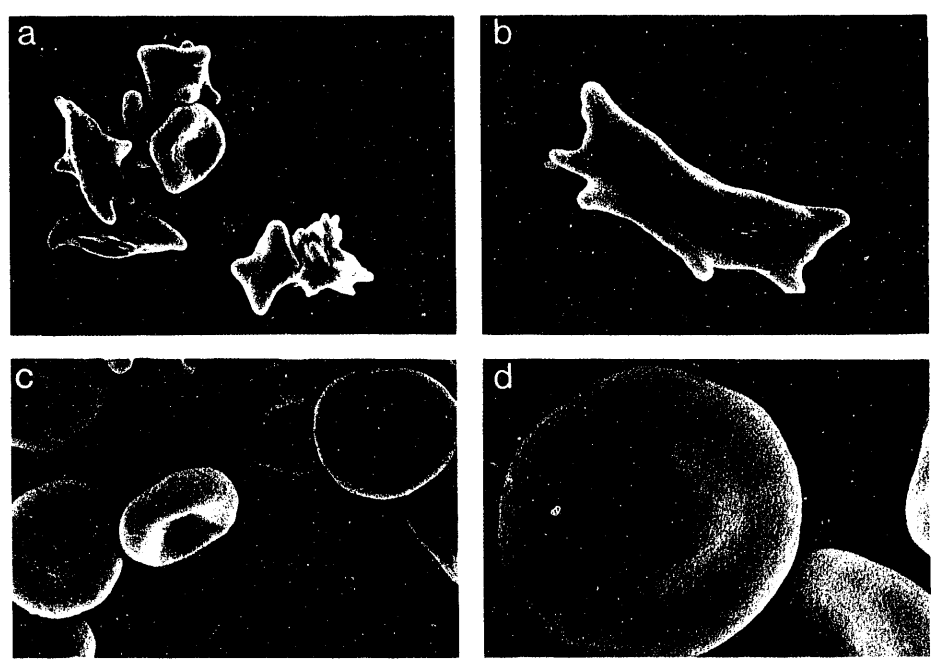
We have found that the carcinogenicity of synthetic pesticides is similar to that of pesticides that plants make naturally to protect themselves from insects and microorganisms. Also similar are the proportions of natural and synthetic chemicals that are positive when tested in rats and mice.

We conclude that prolonged elevation of the rate of mitosis, or cell division, increases mutagenesis—and therefore carcinogenesis. Stimulation of mitosis is likely to be a dominant factor in studies of carcinogenicity that are conducted at high doses.

### Hematopoiesis

Hematopoiesis (the development of blood cells) is a model for the proliferation and differentiation of multipotential stem cells, and thus a natural theme for research into the regulation of gene expression. Blood-cell precursors give rise in the bone marrow to erythrocytes (red blood cells), thrombocytes (blood platelets), and a variety of leukocytes (white blood cells). This process of differentiation and its many ramifications constitute a significant focus of our activities. Specific projects include the study of the hormonal control of erythropoiesis (development of red blood cells), the cloning and molecular characterization of red-cell membrane proteins, studies on the regulation of platelet production, research on the immunologic regulation of hematopoiesis, and the use of transgenic mouse systems to study hemoglobin disorders such as sickle cell anemia.

The work with transgenic mice has borne especially notable results. In particular, we have created the first transgenic organism to carry the human sickle cell gene—a crucial step toward the development of an animal model for sickle cell anemia. Ovum nuclei are injected with a cloned fragment of human DNA that contains the hemoglobin-S gene, which codes for the protein that causes sickle cell anemia. Fertilized eggs that survive a night in culture and divide into two-cell embryos are then implanted into a surrogate mother. Newborn mice are screened for human DNA, and those

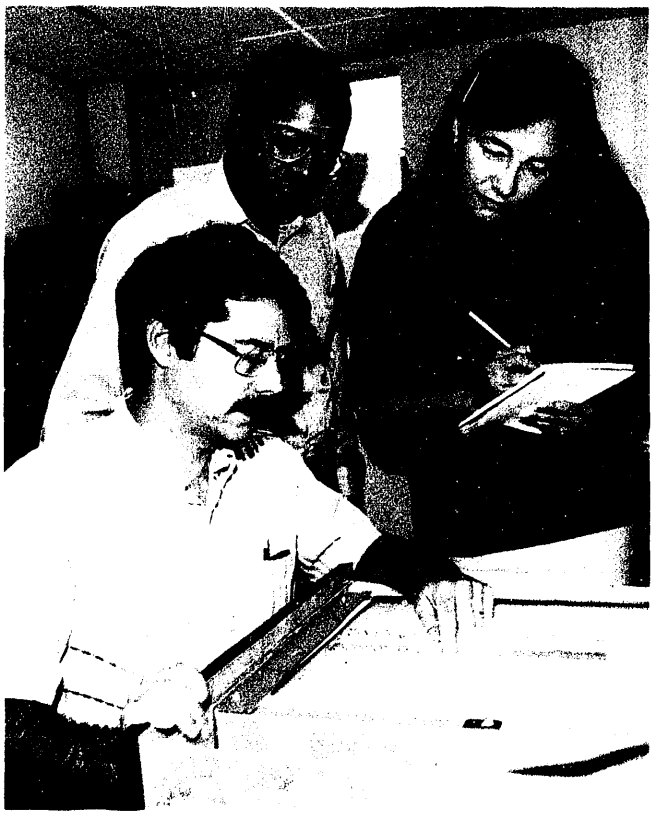



*Transgenic-mouse red blood cells containing human sickling hemoglobin. The cells shown in (a) and (b) are deoxygenated; those in (c) and (d) are oxygenated.*

containing human DNA sequences are then tagged for study. About one out of every 100 eggs injected becomes a transgenic mouse, and about half of the offspring of these mice are also transgenics.

These transgenic mice produce hemoglobin-S, but there is no sickling when their blood cells are oxygenated. All hemoglobin molecules consist of an alpha chain and a beta chain. The interaction between a mutated beta chain and a normal alpha chain leads to pathological polymerization, or linking, of hemoglobin molecules in humans. In mice, however, human beta-S chains interact with normal alpha mouse chains to form functional hemoglobin molecules. Two approaches are being taken to correct this problem. One is to introduce genes that will express human alpha, as well as beta-S, hemoglobin chains in the mouse. The second approach is to genetically engineer a human hemoglobin beta chain that will polymerize more readily than beta-S.

We are also studying protein 4.1, one of the main proteins in the membrane skeleton responsible for maintaining the structural integrity of red blood cells. Protein 4.1 gives the membrane skeleton its great flexibility, allowing a cell about eight microns (millionths of a meter) in diameter to move through capillaries whose diameters are barely three microns. During the past year, we discovered two molecular "switches" that cause structural—and presumably functional—changes in protein 4.1 during erythropoiesis. Also, we are learning how mutations in protein 4.1 cause blood disorders.



*Hematopoiesis research group members John Conboy (left), Mohandas Narla, and Joel Chasis discuss data from their studies of the development and proliferation of blood cells. They are learning how mutations in blood-cell proteins cause blood disorders.*

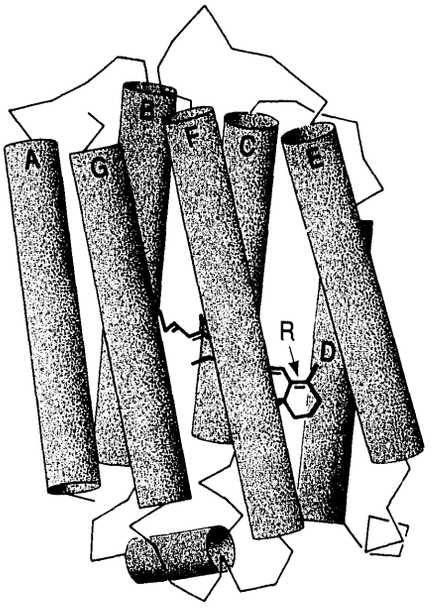
## MACROMOLECULAR STRUCTURE

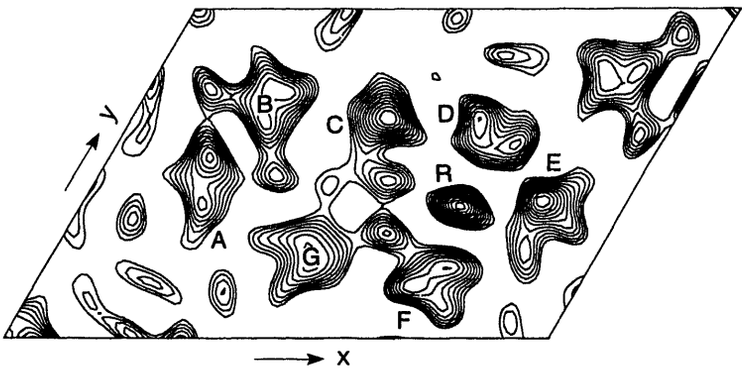
*An understanding of molecular structure and even cellular architecture is an integral part of any complete picture of how a cell's genetic program is regulated and expressed. Accordingly, we are actively pursuing an enhanced structural picture of selected cellular components, emphasizing in particular the use of electron crystallography. One subject of special interest is a family of membrane proteins involved in the selective transport of ions across the cell membrane. For the study of such proteins, electron crystallography has unique appeal, since, unlike x-rays, electrons can be easily focused to produce a high-resolution image, in addition to yielding electron diffraction data. This method also offers a unique opportunity to study membrane proteins in their natural state. We are also using video-enhanced light microscopy and (with members of the Chemical Biodynamics Division) light microscopy with linearly or circularly polarized light. Among applications of the former technique are studies of intracellular transport, where organelles (subcellular structures) can be seen as they move along individual microtubules—minute cylindrical structures found in cells. Our new intermediate voltage electron microscope, which allows viewing of much thicker specimens than can be examined with conventional electron microscopes, is enabling us to study complex structure, including how networks of microtubules and other subcellular components are organized to direct intracellular transport.*

### Electron Crystallography

Electron crystallography achieved a landmark recently with the determination of an atomic model for the structure of a membrane protein, bacteriorhodopsin, from electron diffraction data and high-resolution electron microscope images. This was the first time that the three-dimensional structure of a protein had been determined at the atomic level from electron microscope data. The success of this work—a collaboration of LBL, the Medical Research Council in Cambridge, England, and the Fritz-Haber Institute in Berlin—was greatly aided by use of a computer-controlled spot-scan imaging technique developed at LBL. By reducing blurring, this technique provides a very important improvement in the quality of high-resolution images. The new model for bacteriorhodopsin provides insight into the mechanism by which this protein uses light energy to pump protons across a bacterial cell membrane.

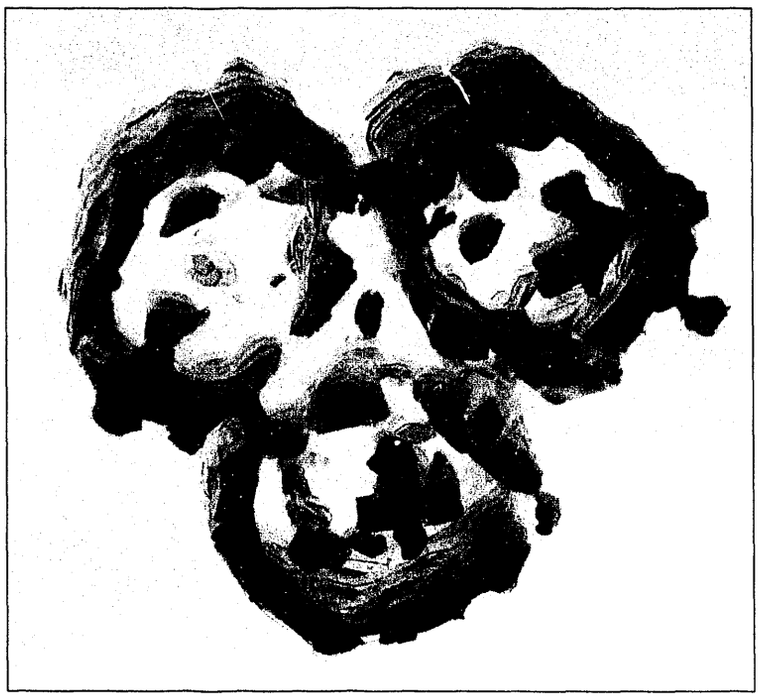
The outer membrane of Gram-negative bacteria, such as *E. coli*, contain a high density of pore-forming proteins, which are often called porins. We are also using electron crystallography to study the structure of one of these, *PhoE*, a porin that is produced under conditions of phosphate starvation and helps transport phosphate-containing compounds and negatively charged molecules into the cell. A three-dimensional structure map has been calculated at a resolution of about 3.5 Å, and we can now gain some idea of the basis of the selectivity and gating of the channel. The channel consists of a cylinder with a wall made up of a β-sheet (an array of amino




*(Left) Schematic drawing of bacteriorhodopsin, a protein that uses light energy to pump protons across a bacterial cell membrane. The cylinders labeled A through G represent helical regions of the protein. The structure labeled R is the retinal molecule that absorbs light. (Below) Cross-section through a 3.5Å map of bacteriorhodopsin. The map, which is based on electron microscope data, shows the density contours of the protein. The retinal molecule appears as a very dense ring.*

acids that contributes to a protein's three-dimensional shape), with a complex structure inside the cylinder. The cylinders occur in groups of three. We are now beginning to interpret the map to determine the path of the amino-acid chain constituting the protein, so that the protein's functional mechanism can be understood in detail.



*3.5Å projection image of* PhoE *porin, an important pore-forming protein found in bacterial membranes. This image was obtained from two-dimensional crystals by means of electron crystallography.*

Procedures for isolation and purification of the cytochrome $bc_1$ protein complex from nonphotosynthetic plant tissues have been fully developed, and cytochrome $bc_1$ from beef heart has been crystallized in a form suitable for x-ray crystallographic study. This protein complex is a link in the electron-transport chain essential to cellular respiration; determination of its structure will provide important information about electron transport in cells.

### Manipulation of a Single DNA Molecule

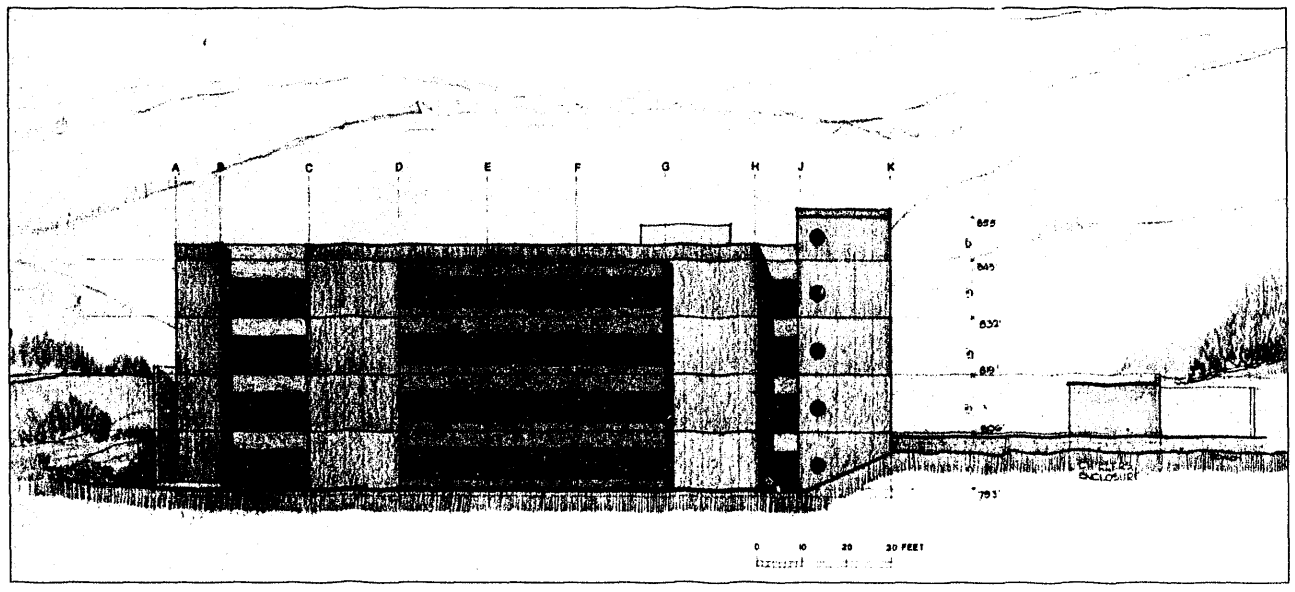
We have invented and constructed a micro-electrophoresis device to manipulate single DNA molecules. The device consists of a network of electrodes with dimensions on the size scale of the length of a single DNA molecule. It uses inhomogeneous electrical fields to enable the placement of different sections of a molecule in specific positions in the electrode net.

In studies of the motion of DNA molecules in the gels used for DNA sequencing, we have observed a number of unexpected effects that may help to explain the anomalous behavior of some DNAs in gels. The acceleration and deceleration of a molecule as it moves around obstacles can be followed and analyzed using computer software which is still under development. We have observed that both the length of the molecule and its velocity vary exponentially with time, and that DNA is apparently much more elastic than had previously been expected.

*Marcos Maestre has developed a new technique that allows him to observe single DNA molecules in motion. On the screen are videotaped images of DNA molecules moving through an electrically charged solution.*

## HUMAN GENOME CENTER

*Since early 1986, the human genome project has emerged from relative obscurity to a place of unique prominence in the minds of both scientists and laypersons. The goal of the project is to determine the sequence of the three billion nucleotide building blocks of the 46 chromosomes found in human cells. The project has gained a widespread scientific commitment unprecedented in the life sciences community, and with its promise of new knowledge about ourselves, it has also captured the public imagination. Coordinated effort on the human genome project began in earnest in 1988, when the U.S. Department of Energy (DOE) designated LBL and the Los Alamos National Laboratory as Human Genome Initiative research centers and provided funding for multidisciplinary research. Activities at LBL's Human Genome Center involve the coordinated efforts of five LBL research divisions: Cell and Molecular Biology, Chemical Biodynamics, Engineering, Information and Computing Sciences, and Materials Sciences—as well as several University of California campuses. A Human Genome Laboratory has been proposed to provide not only the physical space for our growing human genome effort but also the fertile, interactive environment essential to productive multidisciplinary research This integration of molecular genetics, engineering, and computer science into a critical research mass is a key ingredient of the Human Genome Center—and of the DOE's strategy for resource and technology development. The new building, which would provide lab and office space for about 60 professional and support personnel who are now dispersed in several buildings, would also provide a core facility for the common use of expensive instrumentation and technologies. It would thus serve as an important user facility for the biomedical scientific community committed to this DOE-inspired national project.*




*An architect's rendering of the proposed Human Genome Laboratory. This 32,000-square-foot building at LBL would provide the lab and office space needed for the anticipated increase in effort at the Human Genome Center. It would also serve as a user facility for the biomedical scientific community.*

## The Human Genome Project: A Short Overview

One can imagine understanding the human genome at several levels of detail. At the coarsest level, we might seek to determine the whereabouts of the most important human genes—at least to the point that we could assign each to a specific chromosome. This kind of low-resolution mapping has, in fact, now been done for more than a thousand genes. At the next level, the goal might reasonably be to map the genome at some higher resolution—perhaps to establish for every human gene a chromosomal location that is accurate to within a million nucleotides. In this area, we have made the barest start. At a still deeper level lies the "Holy Grail" of biology, the dream of sequencing the human genome. The result would be a string of three billion characters, representing the sequence of nucleotides that defines our species. Included, of course, would be the sequence for every gene, as well as the sequences for the much greater lengths of DNA that have no known function. Should anyone undertake to print it all out, the results would fill 200 volumes the size of the Manhattan phone book.

In a sense, though, even a complete genome sequence is only the beginning of understanding. The deepest mystery is how the potential of 100,000 genes is regulated and controlled, how blood cells and brain cells are able to perform their separate functions with the same apparent genetic program, and how these and countless other cell types arise in the first place from an undifferentiated human embryo. A first step toward solving these subtle mysteries, though, is a more complete physical picture of the master molecules that lie at the heart of it all.

Work at LBL is only part of a broad international effort that will dramatically enhance our understanding of human molecular genetics before the end of this century. In this country, the bulk of the effort will be carried out under the auspices of the DOE and the National Institutes of Health, but significant contributions have already been made both by nonprofit private foundations and by private corporations. The crucial role of the DOE will be to exploit its unique experience in managing large projects and to coordinate multidisciplinary research at its national laboratories—repositories of expertise in physics, engineering, and computer science, as well as the life sciences. The tools and techniques the human genome project will ultimately rely on are thus likely to be developed at laboratories like LBL.

## Philosophy of the Human Genome Center

We feel that "data-producing" efforts and technology innovation should proceed hand in hand, and that both must be tied to specific, interesting biological problems. For example, chromosome mapping targets are chosen because of the potential for finding particular genes, such as the genes for Down's syndrome and familial Alzheimer's on chromosome 21, or for elucidating chromosome function, such as the functions associated with telomeres—the very ends of chromosomes. Large mapping or sequencing efforts that do not allow for technology evolution are almost certain to be obsolete before they are finished, and technology innovation in a vacuum inevitably fails to provide optimal methods for solving biological problems.

Mapping and sequencing methods will undoubtedly continue to evolve rapidly, and ultimately most of the genome will likely be sequenced by methods not in existence today. We must therefore be extremely careful not to invest large amounts of effort, such as would be required for development of commercial-quality software or highly refined hardware, in strategies or tactics that will soon become obsolete. A continual planning process is needed to reevaluate our methods and replace or abandon them as technological advances emerge.

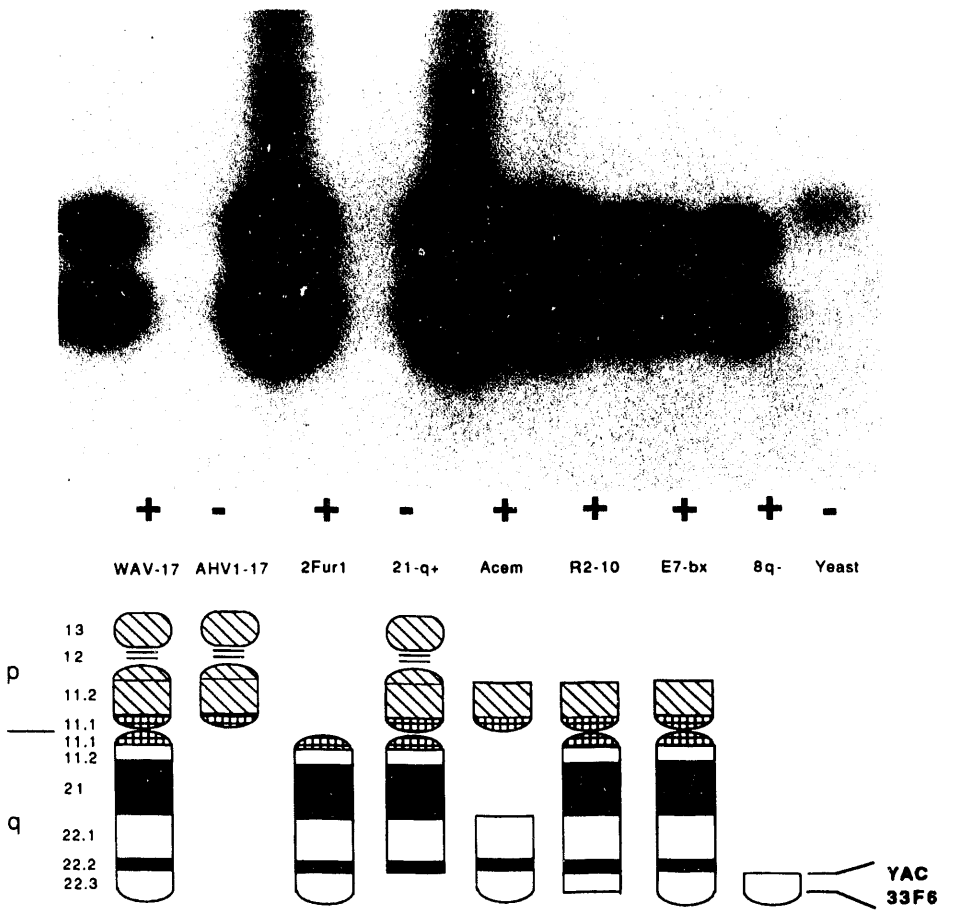
The Human Genome Center both draws on and augments the San Francisco Bay Area's strength in basic biology and human genome research. The concentration of genome project activity in the Bay Area is unequalled at any other U.S. location: Biotechnology companies such as Genentech, Applied Biosystems, and Cetus are conducting genome research, as are Lawrence Livermore National Laboratory, Stanford University, and the University of California's Berkeley and San Francisco campuses. LBL's Genome Center is a significant part of this dynamic research community.

## Mapping

A major goal at the Center has been to map human chromosome 21. In a sense, this effort was the Center's central project, serving not only as a goal in itself but also as a focus for efforts to improve the tools of the trade and to develop new ones.

The basic strategy we are using to map chromosome 21 is to determine the order of large DNA fragments generated by digestion of the chromosome with the restriction enzyme *Not* I. The average fragment is about one million nucleotides in length (because DNA occurs as a double-stranded helix, a fragment would actually contain twice this many nucleotides). The smallest fragments are about 50 thousand nucleotides long. The frequency of the interspersed, highly repeated *alu* sequence (named for the enzyme that cuts it out of a DNA strand) in the human genome is so high that the probability of a 50-thousand-nucleotide DNA fragment being free of *alu* is less than one percent. Since the average-sized *Not* I fragment is much larger than this, it is highly likely that almost all such fragments contain *alu* and can therefore be detected by hybridization with *alu*. In fact, *alu* detects 37 different-sized *Not* I DNA "bands" from chromosome 21 when the fragments are separated by size using pulsed-field gel (PFG) electrophoresis (a technique in which an electric field is used to separate DNA fragments on a two-dimensional gel). Several of these bands contain more than one particular *Not* I fragment, but by combining our results with those of our Japanese collaborators, we can now identify 45 of the 48 DNA fragments estimated to be the minimum constitution of the chromosome. This accounts for 45 million nucleotides, or over 80 percent of the minimum actual length of chromosome 21.

We have also accomplished the key step needed to demonstrate the feasibility of a new strategy for ordering a yeast-artificial-chromosome (YAC) library for chromosome 21. A library is a mechanism for packaging DNA for cloning—that is, replication in large quantities. YACs are replicated in yeast cells, but contain mostly human or other foreign DNA. Because YACs




*Assignment of a yeast artificial chromosome (YAC) to a specific region of human chromosome 21. Two DNA fragments from a human chromosome-21 YAC (33F6) were labeled with a radioisotope and hybridized to DNA from a series of hybrid cell lines that contain all or parts of human chromosome 21. Positive hybridization results occur when the YAC DNA is also present in the cell line. The parts of human chromosome 21 found in the various cell lines are shown pictorially below the hybridization results, which indicate that YAC 33F6 is derived from chromosome-21 band q22.3.*

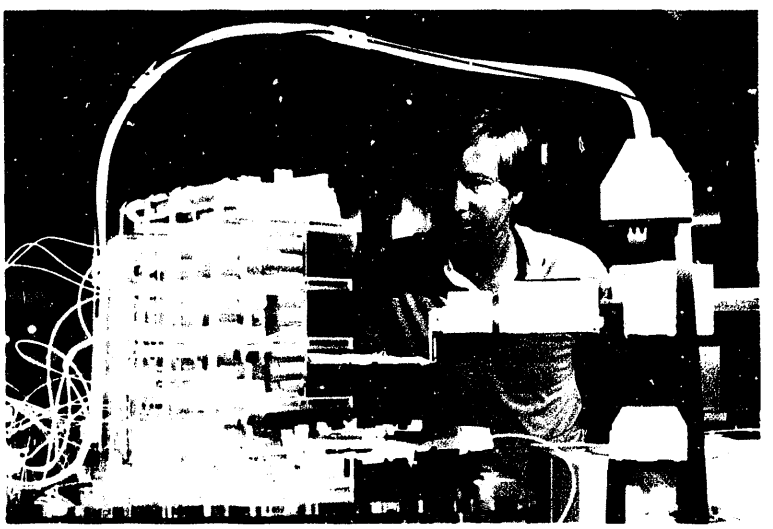
are created randomly, a method is needed for locating ones containing particular genes of interest. Current strategies are slow and inefficient. The new strategy is based on polymerase-chain-reaction probes made from PFG bands. We have demonstrated use of a polymerase chain reaction to amplify only selected human DNA in a slice of PFG-separated *Not* I fragments from a human/rodent hybrid cell line. (The hybrid cells are more efficient to work with than human cells, because the only human chromosome they contain is chromosome 21.) We now plan to use the amplified human DNA as a probe to identify the corresponding YACs. Once these YACs are found, their order will be determined; this process can proceed even before the *Not* I map is finished. Indeed, some of the YACs will prove to contain internal sections of *Not*- I fragments, and these will aid in completion of the map.

### Instrumentation and Automation

We have developed two new methods for detecting DNA in very low concentrations in electrophoresis gels. Both methods rely on laser scanning microscopy for rapid examination of a small volume of fluorescently labeled DNA. The scanning speed and light intensity are adjusted to maxi-

mize the signal from the fluorescent dyes. In one approach, end-labeled DNA fragments run on sequencing gels can be detected at 10 percent of the minimum concentration detected by standard commercial instruments. This potentially provides a tenfold increase in sequencing rate by allowing use of either shorter gels or thinner lanes (the gel columns in which DNA fragments separate into bands). In the second approach, ethidium homodimer is used instead of ethidium to stain a gel. Because the dissociation rate of this dye from DNA is negligible under typical electrophoresis conditions, the DNA can be stained before electrophoresis, eliminating many tedious staining and destaining steps normally done after electrophoresis. The sensitivity of this method for detecting DNA is at least 100 times greater than that of the older ethidium-staining method. This will be a powerful tool in many DNA analytical procedures.

A Hewlett-Packard computer-driven prototype chemical robot was made available to LBL in 1989. The robot consists of an active arm capable of remarkably accurate and precise movement, seven pumps for dispensing and sampling very small volumes of material, and a spectrophotometer for color analysis of the samples. We have demonstrated use of the robot to duplicate arrayed clone libraries in a sterile environment. Arrayed clone libraries, which consist of dozens of clones lined up in rows on a filter, are a major tool in genome mapping because they facilitate screening for clones of interest. However, for such arrays to be effective, they must be replicated accurately and transferred to filters at high density. Currently, at most locations in the world, this extremely tedious work is done manually. The robot has been trained to perform the necessary manipulation of arrayed libraries and is now being tested in a production mode, duplicating two large arrays of interest: a chromosome-21 cosmid library (a library in a bacterial virus, or phage) obtained from Lawrence Livermore National Laboratory and a YAC library from Washington University in St. Louis, Missouri.



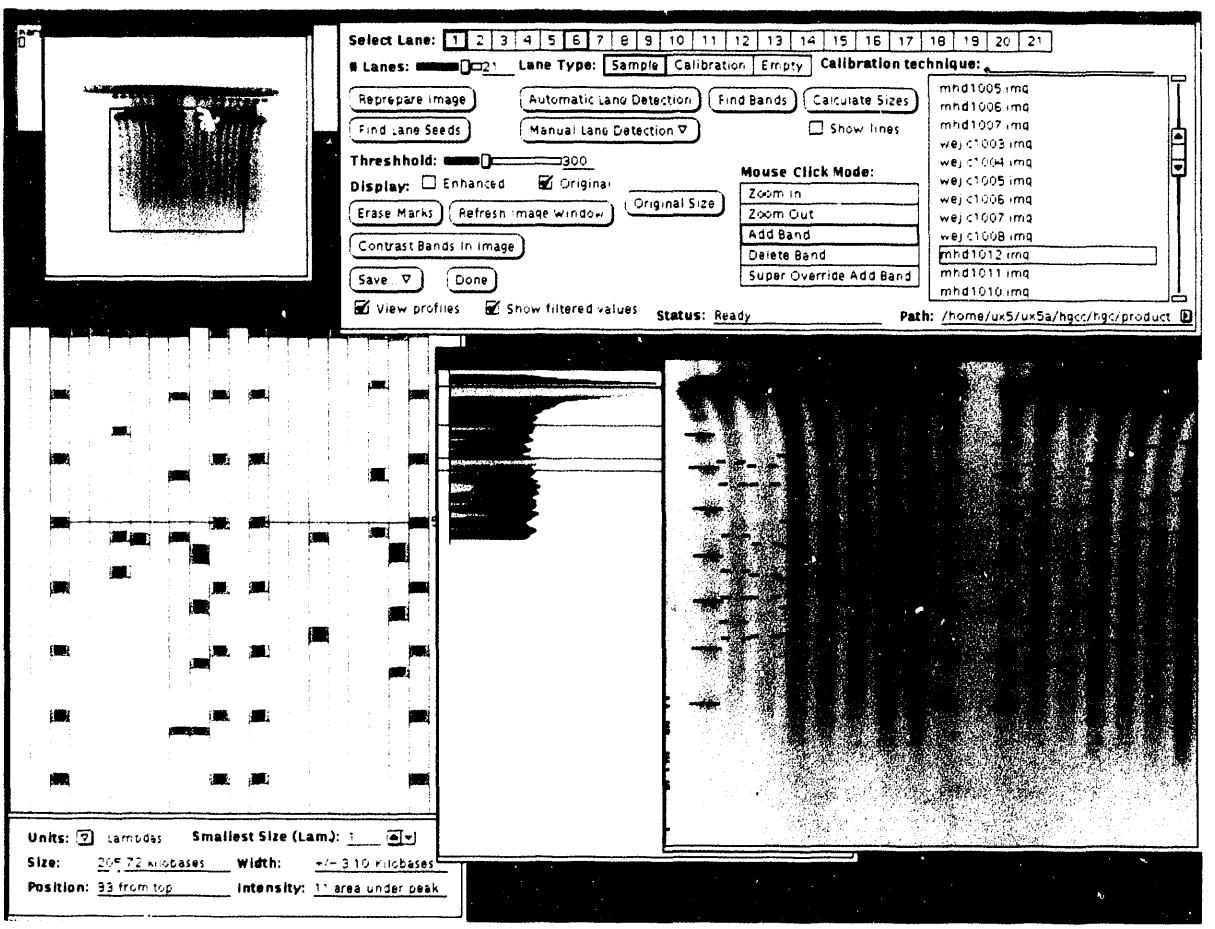
*Hewlett-Packard, Inc., has provided the Human Genome Center with a computer-driven robot for handling and processing biological samples. Application of robotics to speed repetitive tasks in human genome research requires interaction among researchers in a variety of disciplines, including molecular biology, computer science, and engineering. Biologist Jeff Gingrich is shown with the robot.*

Design and construction have been completed for a power supply that allows operation of up to 64 gel boxes with a single IBM PC computer. The system has a 24-electrode array in which individual electrode voltages are selected by the computer, which also determines the timing of changes in the electric-field profiles across the gels. Software has been developed for calculating electrode voltage distributions, given a desired homogeneous or inhomogeneous field profile. A significant advantage of being able to control many gels at once is the ability to establish several experimental plans, which are then automatically documented and recorded as the gels are run.

## Information Handling and Computing

A semiprivate database is being constructed for the community of scientists working on chromosome 21. It is actually a generic physical mapping database: There is nothing that restricts it to chromosome 21. It is designed as a shared database for a small, interacting community and therefore imposes different challenges than would a large, public, fully interactive physical mapping database. The interface that allows a user to interact with the database is currently implemented in Supercard on a Macintosh II computer. The actual database is being implemented in Sybase on a Sun Workstation. The database is accessible to remote users at Keio University in Tokyo and the Eleanor Roosevelt Cancer Center in Denver—two other major chromosome-21 mapping sites—and it can communicate with external public databases such as the Human Gene Mapping Library (HGML) in New Haven, the National Library of Medicine (NLM), and Genbank. It also will eventually be able to communicate directly with private databases such as electronic laboratory notebooks or the files used by robots in clone arraying.

*ANGEL is a program that was developed at LBL to analyze data from electrophoresis separation of DNA fragments. The program automatically identifies DNA lanes (wide vertical lines in lower right window) and centers of bands for particular fragments (short horizontal lines). The lower middle window shows the bands in a single lane after digital filtering to remove noise. Electrophoresis is an essential step in DNA sequencing.*



One of the greatest limitations in much DNA analysis is the need to store, interpret, and compare large numbers of photographic images, typically micrographs or pictures of gels. Because the task of cataloguing these images is formidable, we have constructed an image database that allows the pictures to be stored and searched with relatively simple queries and displayed in whatever combinations are needed. Tools also are available for image enhancement, including background smoothing, intensity analysis, and semiautomatic DNA size determination by comparison with standard samples. Eventually, the database will be provided with a more powerful query structure and with additional image-analysis tools. The image query database will be able to communicate with the chromosome-21 database described above and with electronic laboratory notebooks that fully document the experiments used to create each image.

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## *Cell and Molecular Biology Division Publications*


Ainsworth, E.J., Afzal, S.M.J., Crouse, D.A., Hanson, W.R. and Fry, R.J.M. (1989) Tissue responses to low protracted doses of high-LET radiation or photons: Early and late damage relevant to radio-protection countermeasures. *Adv. Space Res.*, **9(10)**:299-313.

Afzal, S.M.J., Tenforde, T.S., Kavanau, K.S. and Curtis, S.B. (1990) Reoxygenation in rat rhabdomyosarcoma tumor following x-irradiation. *Int. J. Radiat. Oncol. Biol. Phys.*, in press.

Alpen, E.L. and Chester, R.O., eds. (1988) *Population Exposure to the Nuclear Fuel Cycle*. Gorden & Breach Science Publs., NY.

Ames, B.N., Profet, M. and Gold, L.S. (1990) Dietary pesticides (99.99% all natural). *Proc. Natl. Acad. Sci., USA*, **87**:7777-7781.

Ames, B.N. and Gold, L.S. (1990) Chemical carcinogenesis: Too many rodent carcinogens. *Proc. Natl. Acad. Sci., USA*, **87**:7772-7776.

Ames, B.N. and Gold, L.S. (1990) Dietary carcinogens, environmental pollution, and cancer: Some misconceptions. *Med. Oncol. and Tumor Pharmacother.*, 7**(3/4)**:69-85.

Ames, B.N. and Gold, L.S. (1989) Misconceptions regarding environmental pollution and cancer causation. In: *Health Risks and the Press* (Moore, M., ed.) The Media Institute in Cooperation with the American Medical Association, Washington, DC, p19-34.

Ames, B.N. and Gold, L.S. Mitogenesis, mutagenesis and animal cancer tests. In: *Chemically Induced Cell Proliferation: Implications of Risk Assessment* (Butterworth, B. and Slaga, T., eds.) Alan R. Liss, Inc., NY, in press.

Ames, B.N. and Gold, L.S. Perspective: Too many rodent carcinogens: Mitogenesis increases mutagenesis. *Science*, in press.

Ames, B.N., Profet, M. and Gold, L.S. Mutagenesis and carcinogenesis of dietary pesticides (99.99% all natural). In: *Mutagens in Food: Detection and Prevention* (Hayatsu, H., ed.) CRC Press, Inc., Boca Raton, FL, in press.

Ames, B.N., Profet, M. and Gold, L.S. (1990) Nature's chemicals and synthetic chemicals: Comparative toxicology. *Proc. Natl. Acad. Sci., USA.*, **87**:7782-7786.

Auersperg, N., MacLaren, I.A. and Bissell, M.J. (1990) V-K-*ras* transformation induces reversion to an earlier developmental form in adult rat adrenal cells. *Differentiation*, **43(1)**:29-36.

Barcellos-Hoff, M.H., Aggeler, J., Ram, T. and Bissell, M.J. (1989) Functional differentiation and alveolar

morphogenesis of primary mammary cultures on reconstituted basement membrane. *Development*, **105(2):**223-235.

Barcellos-Hoff, M.H. and Bissell, M.J. (1989) A role for the extracellular matrix in the autocrine and paracrine regulation of tissue-specific function. In: *Paracrine and Autocrine Factors in Reproductive Endocrinology* (Gulyas, B.J., McCraken, M. and Krey, L.C., eds.) Plenum Press Publ. Corp., NY, p137-155.

Barcellos-Hoff, M.H. and Bissell, M.J. (1989) Mammary epithelial cells as a model for studies of the regulation of gene expression. In: *Functional Epithelial Cells in Culture* (Matlin, K. and Valentich, J.D., eds.) Alan R. Liss, Inc., NY, p399-433.

Barcellos-Hoff, M.H., Marton, L.J. and Deen, D.F. (1990) Differential drug sensitivity conferred by growth status detected in a mixed population of cycling and noncycling cells. *Cancer Res.*, **50(12):**3551-3555.

Barendsen, G.W., Fabrikant, J.I., Field, S.B., Hahn, F.F., Kellerer, A.M. and Stather, J.W. The RBE of high-LET radiations for nonstochastic effects. *International Commission on Radiological Protection*, Pergamon Press, Oxford, in press.

Basu, A.K., Loechler, E.L., Leadon, S.A. and Essignman, J.M. (1989) Genetic effects of cis-thymine glycol: Site-specific mutagenesis and molecular modeling studies. *Proc. Natl. Acad. Sci., USA*, **86:**7677-7681.

Bates, S.E., Valverius, E., Ennis, B.W., Bronzert, D.A., Sheridan, J.P., Stampfer, M., Lippman, M.E. and Dickson, R.B. Expression of the TGFα/EGF receptor pathway in normal human breast epithelial cells. *Endocrin.*, in press.

Bissell, M.J. and Ram, T.G. (1989) Regulation of functional cytodifferentiation and histogenesis in mammary epithelial cells: Role of the extracellular matrix. *Environ. Health Persp.*, **80:**61-70.

Bissell, M.J. and Wicha, M.S. (1990) Extracellular matrix is required for milk protein gene expression and secretion in mammary epithelial cells. In: *Biology of Lactation* (Houdebine, L.M., ed.) INRA, France, in press.

Blakely, E.A., Chang, P., Lommel, L., Bjornstad, K.A., Dixon, M., Tobias, C., Kumar, K. and Blakely, W.F. (1989) Cell cycle radiation response: Role of intracellular factors. *Adv. Space Res.*, **9(10)**:177-186.

Brillinger, D.A., Downing, K.H. and Glaeser, R.M. (1990) Some statistical aspects of low-dose electron imaging of crystals. *J. Statistical Planning and Inference*. **25**:235-259 (1990).

Brillinger, D.R., Downing, K.H., Glaeser, R.M. and Perkins, G. (1989) Combining noisy images of small crystalline domains in high resolution electron microscopy. *J. Applied Statistics*, **16(2)**:165-175.

Cantor, C.R., Bondopadhyay, S., Bramachari, S.K., Hui, C.-F., McClelland, M., Morse, M. and Smith, C.L. (1988) Torsional stress, unusual DNA structures, and eukaryotic gene expression. In: *Unusual DNA Structures* (Wells, R.D. and Harbey, S.C., eds.) Springer-Verlag, NY, p73.

Cantor, C.R., Caskey, T., Hood, L., Kamely, D. and Omenn, G., eds. *Biotechnology and Human Genetic Predisposition to Disease*. Alan R. Liss, Inc., in press.

Cantor, C.R., Gaal, A. and Smith, C.L. (1988) High resolution separation and accurate size determination in pulsed field gel electrophoresis of DNA; III: Effect of electrical field shape. *Biochem.*, **27**:9216.

Cantor, C.R., Pevny, L. and Smith, C.L. Organization and evolution of genomes as seen from a megabase perspective. *Proc. of the NATO/FEBS Workshop*, Spetsai, Greece. Plenum Press, NY, in press.

Cantor, C.R. and Smith, C.L. Large DNA technology and possible applications in gene transfer. In: *Gene Transfer in Animals:* UCLA Symp. on Molecular and Cellular Biology, (Verma, I., Mulligan, R. and Beaudet, A., eds.) New Series 87(I), in press.

Cantor, C.R. and Smith, C.L. (1988) Mapping the genome. In: *Biotechnology and the Human Genome: Innovations and Impact* (Woodhead, A.D. and Barnhart, B.J., eds.) Plenum Press, NY, p11.

Cantor, C.R. and Smith, C.L. Perspectives on the human genome project. In: *Biotechnology and Human Genetic Predisposition to Disease (Cantor, C., Caskey, T., Hood, L., Kamely, D. and Omenn, G., eds.) UCLA Symp. on Molecular and Cellular Biology, New Series 126, Alan R. Liss, Inc., NY, in press.*

Cantor, C.R. and Smith, C.L. Structure and function of nucleic acids and proteins. Taipei Symposium, in press.

Cantor, C.R., Smith, C.L. and Mathew, M.K. (1988) Pulsed-field gel electrophoresis of very large DNA

molecules. In: *Annual Review of Biophysics and Biophysical Chemistry* (Engelman, D.M., Cantor, C.R. and Pollard, T.D., eds.) Annual Reviews, Inc., Palo Alto, CA, p287.

Chasis, J.A., Prenant, M., Leung, A. and Mohandas, N. (1989) Membrane assembly and remodeling during reticulocyte maturation, **74(3)**:1112-1120.

Chatterjee, A. (1989) Radical-induced DNA damage and specific implications in evaluating genetic changes. *Free Radical Res. Comm.*, **6(2-3)**:189-190.

Chatterjee, A. (1989) Radiobiological effects of high-LET particles: DNA strand breaks. *Nucl. Instruments and Methods in Physics Res.*, **A280**:439-448.

Chatterjee, A. and Blakely, E. (1989) Applications of the BEVALAC in biophysical research. *Nucl. Instruments and Methods in Physics Res.*, **B40/41**:1365-1371.

Chatterjee, A. and Holley, W.R. Biochemical mechanisms and clusters of damage of high-LET radiation. *Adv. Space Res.*, in press.

Chatterjee, A. and Holley, W.R. (1989) Energetic electron tracks and DNA strand breaks. *Nucl. Tracks and Radiat. Meas.*, **16(213)**:127-133.

Chatterjee, A. and Holley, W.R. (1990) Energy deposition mechanisms and biochemical aspects of DNA strand breaks by ionizing radiation. *Int. J. Quantum Chem.*, in press.

Chatterjee, A. and Holley, W.R. (1990) A general theory of DNA strand break production by direct and indirect effects. *Radiat. Prot. Dosimetry*, in press.

Chatterjee, A. and Llacer, J. (1989) Applications of radioactive beams in diagnostic studies. In: *Proc. of the 1st International Conf. on Radioactive Nucl. Beams*, Berkeley, CA.

Chen, L-H. and Bissell, M.J. (1989) A novel regulatory mechanism for whey acidic protein gene expression. *Cell Regulation*, **1**:45-54.

Cheng, J.F., Smith, C.L. and Cantor, C.R. (1989) Isolation and characterization of a human telomere. *Nucl. Acids Res.*, **17(15)**:6109-6127.

Clemons, G.K. Comparison of radioimmunoassay of erythropoietin. In: *Humoral and Cellular Regulation of Erythropoiesis* (Zanjani, A., Tavassoli, M. and Ascensao, J.L., eds.) Scientific Press, NY, in press.

Cole, G.M. and Mortimer, R.K. (1989) Failure to induce a DNA repair gene, RAD54, in *Saccharomyces cerevisiae* does not affect DNA repair or recombination phenotypes. *Mol. and Cell. Biol.*, **9(8)**:3314-3322.

Cole, G.M., Schild, D. and Mortimer, R.K. (1989) Two DNA repair and recombination genes in *Saccharomyces cerevisiae*, RAD52 and RAD54, are induced during meiosis. *Mol. and Cell. Biol.*, **9(7)**:3101-3104.

Conboy, J.J. and Hotchkiss, J.H. (1989) Photolytic interface for high-performance liquid chromatography—chemiluminescence detection of non-volatile N-nitroso compounds. *Analyst*, **114(2)**:155-159.

Couto, L., Spangler, E. and Rubin, E.M. (1989) A method for the introduction of high molecular weight DNA into mouse embryos. *Nucl. Acids Res.*, **17**:8010.

Curtis, S.B. Ionization clusters and their proximity as relevant physical lesions. *Proc. of the 10th Symp. on Microdosimetry, Rome, Italy* (in press).

Curtis, S.B. (1989) The Katz cell-survival model and beams of heavy charged particles. *Nucl. Tracks and Radiat. Meas.* **16(2/3)**:97-103.

Curtis, S.B. (1989) A possible role of the inverse dose-rate effect in the radon exposure problem. In: *Low Dose Radiation: Biological Bases of Risk Assessment* Baverstock, K.F. and Stather, J.W., eds.) Taylor and Francis, London, p547-553.

Curtis, S.B. and Thames, H.D. (1989) Variation in $\alpha/\beta$ based on available time for repair. *Brit. J. Radiol. Report*, **19**:17-19.

Daban, J.-R. and Cantor, C.R. (1989) Use of fluorescent probes to study nucleosomes. *Methods of Enzymol.*, **170**:192-214.

Deen, D.F., Morgan, W.F., Tofilon, P.J. and Barcellos-Hoff, M.H. (1989) Measurement of sister chromatid exchanges and their relationship to DNA damage, repair and cell killing. *Pharma. and Therapeutics*, **42(3)**:349-360.

Dosanjh, M.K., Essignmann, J.M., Goodman, M.F. and Singer, B. (1990) Comparative efficiency of forming m4T.G base pairs at a unique site using *Escherichia coli* DNA polymerase a-primase complex. *Biochem.*, **29**:4698-4703.

Durante, M., Grossi, G., Yang, T.C. and Roots, R. (1990) Genetic changes in mammalian cells transformed by helium ions. *Adv. Space Res.*, in press.

Esposito, M.S. and Brown, J.T. (1990) Conditional hyporecombination mutants of three REC genes of *Saccharomyces cerevisiae*. *Current Genetics*, **7**:7-12.

Euteneuer, U., Haimo, L.T. and Schliwa, M. (1989) Microtubule bundles of reticulomyxa networks are of uniform polarity. *Eur. J. Cell Biol.*, **49**:373-376.

Euteneuer, U., Johnson, K., Koonce, M.P., McDonald, K.L., Tong, J. and Schliwa, M. (1989) *In vitro* analysis of cytoplasmic organelle transport. In: *Cell Movement*; Kinesis, Dynein, and Microtubule Dynamics (Warner, F.D. and McIntosh, J.R., eds.) Alan R. Liss, Inc., NY, **2**:155-167.

Euteneuer, U., Johnson, K.B. and Schliwa, M. (1989) Photolytic cleavage of cytoplasmic dynein inhibits organelle transport in reticulomyxa. *Eur. J. Cell Biol.*, **50**:34-40.

Fan, J.-B., Chikashige, Y., Smith, C.L., Niwa, O., Yanagida, M. and Cantor, C.R. (1989) Construction of a *Not* I restriction map of the fission yeast *Schizosaccharomyces pombe* genome. *Nucl. Acids Res.*, **17(7)**:2801-2818.

Foiles, P.C., Singer, B. and Miglietta, L.M. (1990) Development of a monoclonal antibody specific for the vinyl chloride-DNA adduct N2,3-ethenodeoxy-guanosine and the detection of this adduct in V79 cells. *Proc. Am. Assoc. Cancer Res.*, **31**:91.

Folkman, W., Kusmierek, J.T. and Singer, B. (1990) A new one-step method for the preparation of 3′,5′-bisphosphates of acid labile deoxynucleosides. *Chem. Res. Toxicol.* **3.**

Fraenkel-Conrat, H. (1990) A tobacco mosaic virus-hybrid expresses and loses an added gene chemtract. *Biochem. and Mol. Biol.*, **1**:171-173.

Fraenkel-Conrat, H. (1988) Tobacco necrosis, satellite tobacco necrosis, and related viruses. *The Plant Viruses* (Koenig, R., ed.) Plenum Publishing Corp.,**Vol 3**.

Fraenkel-Conrat, H. (1990) Virus reconstitution and the proof of the existence of genomic RNA. *Bioessays*, **12**:1-2.

Game, J.C., Sitney, K.C., Cook, V.E. and Mortimer, R.K. (1989) Use of a ring chromosome and pulsed-field gels to study interhomolog recombination, double-strand DNA breaks and sister-chromatid exchange in yeast. *Genetics*, **123(4)**:695-713.

Gibbs, E.J., Tinoco Jr., I., Maestre, M.F., Ellinas, P.A. and Pasternak, R.F. (1988) Self-assembly of porphyrins on nucleic acid templates. *Biochem. Biophys. Res. Comm.*, **157(1)**:350-358.

Glaeser, R.M. and Ceska, T.A. (1989) High-voltage electron diffraction from bacteriorhodopsin (purple membrane) is measurably dynamical. *Acta Cryst.*, **A45**:620-628.

Glaeser, R.M., Tong, L. and S.-H. Kim. (1989) Three-dimensional reconstructions from incomplete data: Interpretability of density maps at 'atomic' resolution. *Ultramicroscopy*, **27**:307-318.

Gold, L.S. and Ames, B.N. (1990) The importance of ranking possible carcinogenic hazards using HERP. *Risk Analysis*, in press.

Gold, L.S., Bernstein, L., Magaw, R. and Slone, T.H. (1989) Interspecies extrapolation in carcinogenesis: Prediction between rats and mice. *Environ. Health Persp.*, **81**:211-219.

Gold, L.S., Slone, T.H., Backman, G.M., Eisenberg, S., Da Costa, M., Wong, M., Manley, N.B., Rohrbach, L. and Ames, B.N. (1990) 3rd chronological supplement to the carcinogenic potency database: Standardized results of animal bioassays published through December 1986 and by the National Toxicology Program through June 1987. *Environ. Health Persp.*, **84:**215-286.

Gold, L.S., Slone, T.H. and Bernstein, L. (1989) Summary of carcinogenic potency ($TD_{50}$) and potency database. *Environ. Health Persp.*, **79:**259-272.

Goodwin, E., Blakely, E.A., Ivery, G. and Tobias, C. (1989) Repair and misrepair of heavy-ion-induced chromosomal damage. *Adv. Space Res.*, **9(10)**:83-89.

Goth-Goldstein, R. and Johnson, P.I. (1990) Repair of alkylation damage in *Saccharomyces cerevisiae*. *Mol. and Gen. Genetics*, **221**:353-357.

Hall, E. (Chap. ed.), Astor, M., Bedord, J., Borek, C., Curtis, S.B., Fry, R.J.M., Geard, G., Hei, T., Mitchell, J., Oleinick, N., Rubin, J., Tu, A., Ullrich, R., Waldren, C. and Ward, J. (1988) Basic radiobiology. *Am. J. Clin. Oncol.*, **11(3)**:220-252.

Halmesmaki, E., Teramo, K.A., Widness, J.A., Clemons, G.K., Ylikorkala, O. (1990) Maternal alcohol abuse is associated with elevated fetal erythropoietin levels. *Obstetrics and Gynecology*, **76**:(2)219-222.

Henderson, R., Baldwin, J.M., Ceska, T.A. Zemlin, F., Beckmann, E. and Downing, K.H. (1990) Model for the structure of bacteriorhodopsin based on high-resolution electron cryo-microscopy. *J. Mol. Biol.*, **213(4)**:899-929.

Holley, W.R. and Chatterjee, A. (1990) The application of chemical models to cellular DNA damage. *NATO ASI Series*, in press.

Holley, W.R., Chatterjee, A. and Magee, J.L. (1990) Production of DNA strand breaks by direct effects of heavy charged particles. *Radiat. Res.*, **121(2)**:161-168.

Hosobuchi, M. and Stampfer, M. (1989) Effects of transforming growth factor β on growth of human mammary epithelial cells in culture. *In Vitro*, **25**:705-713.

Houseal, T.W., Bustamonte, C., Stump, R.F. and Maestre, M.F.(1989) Real time imaging of single DNA molecules with fluorescence microscopy. *Biophys. J.*, **56**:507-516.

Huang, L.S. and Berry, E.A. (1990) Purification and characterization of the proton translocating plasma membrane ATPase of red beet storage tissue. *Biochim. Biophys. Acta*, **1039(2)**:241-252.

Jap, B.K. (1989) Molecular design of *PhoE* Porin and its functional consequences. *J. Mol. Biol.*, **205**:407-419.

Jap, B.K., Downing, K.H. and Walian, P.J. (1990) Structure of *PhoE* Porin in Projection at 3.5Å Resolution. *J. Struct. Biol.*, **103**:57-63.

Jap, B.K. and Walian, P.J. Biophysics of structure and function of porins. *Quart. Rev. Biophys.*, in press.

Jap, B.K., Walian, P.J. and Downing, K.H. (1989) Image of *PhoE* Porin in projection at 3.5Å resolution. *Proc. of the 47th Annual Meeting of the Electron Microscopy Society of America*, San Francisco Press, p818-819.

Johnston, J.R., Curran, L., Contopoulou, R.C. and Mortimer, R.K. (1989) *Yeast*, **5**:255-259.

Jukes, T.H. (1990) From RNA viruses to *Australopithecus*. *J. Mol. Evolution*, **30(6)**:477-478.

Jukes, T.H (1990) Random walking. *J. Mol. Evolution*, **30(5)**:225-239.

Keller, R.W., Dunlap, D.D., Bustamonte, C., Keller, D.J. and Maestre, M.F. (1990) Scanning tunneling microscopy images of metal-coated bacteriophages and uncoated, double-stranded DNA. *J. Vacuum Sci. and Tech.* A-Vacuum Surfaces and Films V8 **1**:706-712.

Kipp, D.E. and Schwarz, R.I. Effectiveness of isoascorbate versus ascorbate as an inducer of collagen synthesis in primary avian tendon cells. *J. Nutri. Sci.*, in press.

Kitenake, T., Gilbert, R.D., Alonso, J., Siu, B.L., Clemons, G.K. and Longo, L.D. (1989) Fetal cardiovascular and endocrine responses to long-term hypoxemia. *Am. J. Physiol.*, **256**:R1348-1354.

Kokko, N., Clemons, G.K., and Lomax, P. The relationship between the temperature and endocrine changes induced by cholinesterase inhibitors. *Pharmacol.*, in press.

Kronenberg, A. (1989) Molecular characterization of thymidine kinase mutants of human cells induced by densely ionizing radiation. *Mutation Res.*, **211**:215-224.

Kronenberg, A. and Blakely, E.A. (1989) Locus specificity of mutation in human lymphoblastoid cells: LET effects. In: *Cell Transformation and Radiation-Induced Cancer in Man* (Chadwick, K.H., Seymour, C., Barnhart, B. and Hilgar, A., eds.) Bristol, p215-222.

Kronenberg, A. and Little, J.B. (1989) Locus specificity for mutation induction in human cells exposed to accelerated heavy ions. Int. *J. Radiat. Biol.*, **55**:913-924.

Kronenberg, A. and Little, J.B. (1989) Mutagenic properties of low doses of x-rays, fast neutrons, and selected heavy ions in human cells. In: *Low Dose Radiation - Biological Bases of Risk Assessment* (Baverstock, K.F. and Stather, J.W., eds) Taylor and Francis, p554-559.

Kuhlbrandt, W. and Downing, K.H. (1989) Two-dimensional structure of plant light-harvesting complex at 3.7Å resolution by electron crystallography. *J. Mol. Biol.*, **207**:823-828.

Kusmierek, J.T., Folkman, W. and Singer, B. (1989) Synthesis of N,23-ethenodeoxyguanosine-5'-phosphate, and N,23-ethenodeoxyguanosine-5'-triphosphate: Stability of the glycosyl bond in the monomer and in poly[dG,edG-dC]. *Chem. Res. in Toxicol.*, **2**:230-233.

Larabell, C.A. and Chandler, D.E. (1990) Stepwise transformation of the vitelline envelope of *Xenopus* eggs at activation: A quick-freeze, deep-etch analysis. *Dev. Biol.*, **139(2)**:263-268.

Larabell, C.A. and Chandler, D.E. (1989) Quick-freeze, deep-etch, rotary-shadow views of the extracellular matrix and cortical cytoskeleton of *Xenopus laevis* eggs. *J. Electron Microscopy Tech.*, **13(3)**:228-243.

Larabell, C. and Chandler, D.E. (1989) The coelomic envelope of *Xenopus laevis* eggs: A quick-freeze, deep-etch analysis. *Dev. Biol.*, **131(1)**:126-135.

Leadon, S.A. Differential repair of DNA damage in specific DNA sequences of human cells. In: *Transformation of Human Diploid Fibroblasts* (Milo, G.E., ed.) CRC Press, Inc., Boca Raton, FL, in press.

Leadon, S.A. (1988) Molecular mechanisms involved in the production and repair of DNA damage. *EPRI Workshop on Ionizing Radiation Effects*, San Diego, CA.

Leadon, S.A. (1988) Workshop report from the Division of Research Grants, National Institutes of Health: "Oxy radicals in carcinogenesis-A chemical pathology study section workshop." *Cancer Res.*, **48**:382-387.

Leadon, S.A. and Snowden, M.M. (1988) Differential repair of DNA damage in the human metallothionein gene family. *Mol. and Cell. Biol.*, **8(12)**:5331-5338.

Leibson, C., Brown, M., Thibodeau, S., Stevenson, D., Vreman, H., Clemons, G., Callen, W. and Moore, L.G. (1989) Neonatal hyperbilirubinemia at high altitude: Role of increased bilirubin production and delayed clearance. *Am. J. Dis. Chil.*, **143**:983-987.

Lett, J.T., Cox, A.B., Story, M.D., Ehmann, U.K. and Blakely, E.A. (1989) Responses of synchronous L5178Y S/S cells to heavy ions and their significance for radiobiological theory. *Proc. Royal Society*, London, **237(1286)**:27-42, in press.

Llacer, J., Schmidt, J.B. and Tobias, C.A. (1990) Characterization of fragmented heavy-ion beams using a three-stage telescope detector: Detector configuration and instrumentation. *Med. Phys.*, **17(2)**:158-162.

Llacer, J., Schmidt, J.B. and Tobias, C.A. (1990) Characterization of fragmented heavy-ion beams using a three-stage telescope detector: Measurements of 670-MeV/amu $^{20}$Ne beams. *Med. Phys.*, **17(2)**:151-157.

Mangum, F.I., Berry, E.A., Parikh, U.K. and Ladd, D. (1990) Optimal etching time of glass ionomer cement for maximum bond of composite resin. *J. Am. Dental Assoc.*, **120(5)**:535-539.

Mangum, F.I. and Berry, E.A. An *in vitro* study of the effectiveness of bonding agents in preventing microleakage in composite restorations of erosion and abrasion lesions. *Gen. Dentistry*, **37(1)**:26-28.

Martins-Green, M. Transmission electron microscopy and immunolabelling of tissues for light and electron microscopy. In: *Post-Implantation Mammalian Embryo: A Practical Approach*, (Copp, A. and Cockroft, D., eds.) IRL Press, in press.

Martins-Green, M. and Bissell, M.J. (1990) Localization of 9E3/CEF-4 in avian tissues: Expression is absent in Rous sarcoma virus-induced tumors but is stimulated by injury. *J. Cell. Biol.*, **110(3)**:581-595.

Mathew, M.K., Hui-C-F., Smith, C.L. and Cantor, C.R. (1988) High-resolution separation and accurate size determination in pulsed field gel electrophoresis of DNA; I: DNA size standards and the effect of agarose and temperature. *Biochem.*, **27(26)**:9204-9210.

Mathew, M.K., Smith, C.L. and Cantor, C.R. (1988) High-resolution separation and accurate size determination in pulsed field gel electrophoresis of DNA; II: Effect of pulse time and electric field strength and implications for models of the separation process. *Biochem.*, **27**:9210.

Mathew, M.K., Hui-C-F., Smith, C.L. and Cantor, C.R. (1988) High-resolution separation and accurate size determination in pulsed field gel electrophoresis of DNA; IV: The influence of DNA topology. *Biochem.*, **27**:9222.

McKenney, J., Valeri, C.R., Mohandas, N., Fortier, N., Giorgio, A., and Snyder, L.M. (1990) Decreased *in vivo* survival of hydrogen peroxide-damaged baboon red blood cells. *Blood*, **76(1)**:206-211.

Mendonca, M.S., Rodriguez, A. and Alpen, E.L. (1990) Differential expression of potentially lethal damage in exponentially growing and quiescent 9L cells. *Radiat. Res.*, **122**:38-43.

Mendonca, M.S., Rodriguez, A. and Alpen, E.L. (1989) Quiescence in 9L cells and correlation with radiosensitivity and PLD repair. *Radiat. Res.*, **117**:443-447.

Merrill, D.W. (1989) Integrated databases in environmental health applications. In: Proc. of Environ. Databases Workshop Reviewed in *Health and Environ. Digest*, **3(8)**:8-9.

Miller, K.L., Schooley, J.C., Smith, K.L., Kullgren, B., Mahlmann, L.J. and Silverman, P.H. (1989) Inhibition of erythropoiesis by a soluble factor in murine malaria. *Exp. Hematol.*, **17(4)**:379-385.

Miller, K.L., Silverman, P.H., Kullgren, B. and Mahlmann, L.J. (1989) Tumor necrosis factor alpha and the anemia associated with murine malaria. *Infection and Immunity*, **57(5)**:1542-1546.

Mohandas, N., Johnson, A., Wyatt, J., Croisille, L., Reeves, J., Tycko, D., and Groner, W. (1989) Automated quantitation of cell density distribution and hyperdense cell fraction in RBC disorders. *Blood*, **74(1)**:442-447.

Mohandas, N. and Evans, E. (1989) Rheologic and adherence properties of sickle cells: Potential contribution to hematologic manifestations of the disease. *Annals New York Acad. Sci.*, **565**:327-337.

Mortimer, R.K., Schild, D., Contopoulou, C.R. and Kans, J.A. (1989) Genetic map of *Saccharomyces cerevisiae*, Edition 10. *Yeast*, **5(5)**:321-403.

Ohama, T., Osawa, S., Watanage, K. and Jukes, T.H. (1990) Evolution of the mitochondrial genetic code; IV: AAA as an asparagine codon in some animal mitochondria. *J. Mol. Evolution*, **30(4)**:329-332.

Parkins, C.S., Rodriguez, A. and Alpen, E.L. (1989) Radiation damage to glucose concentrating capacity and cell survival in kidney tubule cells: Effects of fractionation. *Int. J. Radiat. Biol.*, **55**:15-26.

Parry, G., Beck, J.C., Moss, L.M., Bartley, J. and Ojakian, G.K. (1990) Determinants of apical membrane polarity in mammary epithelial cell cultures: The role of cell-cell, cell-substratum, and membrane cytoskeleton interactions. *Exp. Cell Res.*, **188**:302-311.

Patel, D., Singer, B. and Strauss, B.S., eds. (1990) Meeting report: Molecular events in mutation and cancer (AACR special conference in cancer research). *Cancer Res.*, **50**:2853-2856.

Pasvol, G., Chasis, J.A., Mohandas, N., Anstee, D.J., Tanner, M.J. and Merry, A.H. (1989) Inhibition of malarial parasite invasion by monoclonal antibodies against glycophorin A correlates with reduction in red cell membrane deformability. *Blood*, **74(5)**:1836-1843.

Pavlovic-Kentera, V., Clemons, G.K., Trbojevic, S., Dimkovic, N., Djukanovic, L. (1990) Erythropoietin and anemia in the progression of Balkan endemic nephropathy and other renal disease. *Nephron*, **54**(2):139-143.

Perciballi, M., Conboy, J.J. and Hotchkiss, J.H. (1989) Nitrite-cured meats as a source of endogenously and exogenously formed N-nitrosoproline in the ferret. *Food and Chemical Tech.*, **27(2)**:111-116.

Preston, B.D., Zakour, R.A., Singer, B. and Loeb, L.A. (1988) Fidelity of base selection by DNA polymerase: Studies on site-specific incorporation of base analogues. In: *DNA Replication and Mutagenesis* (Moses, R.E. and Summers, W.C., eds.) Am. Soc. Microbiol., Washington, DC, p196-207.

Racowsky, C., Baldwin, K.V., Larabell, C.A., DeMarais, A.A. and Kazilek, C.J. (1989) Down-regulation of membrane granulosa cell gap junctions is correlated with irreversible commitment to resume meiosis in golden Syrian hamster oocytes. *Eur. J. Cell Biol.*, **49(2)**:244-251.

Ramirez, J.A., Vacata, V., McClusker, J.H., Haber, J.E., Mortimer, R.K., Owen, W.G. and Lecar, H. (1989) ATP-sensitive $K^+$ channels in a plasma membrane $H^+$-ATPase mutant of the yeast *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci., USA*, **86(20)**:7866-7870.

Reid, M.E., Takakuwa, Y., Conboy,. J., Tchernia, G. and Mohandas, N. (1990) Glycophorin C content of human erythrocyte membrane is regulated by protein 4.1. *Blood*, **75**:2229.

Reid, M.E., Takakuwa, T., Tchernia, G., Jensen, R.H., Chasis, J.A. and Mohandas, N. (1989) Functional role for glycophorin C and its interaction with the human red cell membrane skeletal component, protein 4.1. *Progress in Clinical and Biological Res.*, **319**:553-71, discussion 572-573.

Robinson, W.P., Kramer, P., Payami, H., Motro, U., Esposito, M.S. and Thompson, G. (1989) Disease associations and disequilibrium mapping. In: *Progress and Clinical and Biological Res.* (Elston, R., Hodge, S., Spence, A., MacCluer, J. and Liss, A.R., eds.), p57-62.

Roots, R., Henle, E., Holley, W.R. and Chatterjee, A. (1990) Measurements of released nucleobases after gamma-irradiation of DNA in solution in air. *Radiat. Res.*, in press.

Roots, R., Holley, W., Chatterjee, A., Irizarry, M. and Kraft, G. (1990) The formation of strand breaks in DNA after high-LET irradiation: A comparison of data from *in vitro* and cellular systems. *Int. J. Radiat. Biol.*, **58(1)**:55-69.

Roots, R., Holley, W., Chatterjee, A., Rachal, E. and Kraft, G. (1989) The influence of radiation quality on the formation of DNA breaks. *Adv. Space Res.*, **9(10)**:45-55.

Rubin, E.M., Andrews, K. and Kan, Y.W. (1989) Newborn screening by DNA analysis of dried blood spots. *Hum. Genet.*, **82**:134-136.

Saffran, W., Welsh, J., Knobler, R., Gasparro, F., Cantor, C. and Edelson, R. (1988) Preparation and characterization of biotinylated psoralen. *Nucl. Acids Res.*, **16**:7221.

Salomon, D.S., Kidwell, W.R., Kim, N., Ciardiello, F., Bates, S.E., Valverius, E.M., Lippman, M.E., Dickson, R.B. and Stampfer, M. (1989) Modulation by estrogen and growth factors of transforming growth factor-alpha and epidermal growth factor receptor expression in normal and malignant human mammary epithelial cells. *Recent Results in Cancer Res.*, **113**:57-69.

Sanengen, T., Clemons, G.K., Halvorsen, S. and Widness, J.A. (1989) Immunoreactive erythropoietin and erythropoiesis stimulating factor(s) in plasma from hypertransfused neonatal and adult mice. *Acta. Physiol. Scand.*, **135**:11-16.

Schimmerling, W. and Curtis, S.B. (1989) Biomedical and space-related research with heavy ions at the BEVALAC (Workshop Summary) *Radiat. Res.* **119**:193-204.

Schliwa, M. (1989) Head and Tail. *Cell*, **56**:719-720.

Schmidhauser, C., Casperson, G.F., Myers, C.A. and Bissell, M.J. (1990) Extracellular matrix and hormones regulate β-casein gene 5′ sequences. In: *Proc. of the 2nd Pezcoller Foundation Symp.*, Centro Italiano Congressi, Rome, Italy, in press.

Schrier, S.L., Rachmilewitz, E. and Mohandas, N. (1989) Cellular and membrane properties of alpha and beta thalassemic erythrocytes are different: Implication for differences in clinical mani-festations. *Blood*, **74(6):**2194-2202.

Shavers, M.R., Curtis, S.B., Miller, J. and Schimmerling, W. (1990) The fragmentation of a 670A-Me V neon-20 as a function of depth in water. II. One generation transport theory. *Radiat. Res.*, in press.

Sieweke, M.H., Stoker, A.W. and Bissell, M.J. (1990) Evaluation of the cocarcinogenic effect of wounding in Rous sarcoma virus tumorigenesis. *Cancer Res.*, **49(22):**6419-6424.

Sieweke, M.H., Thompson, N.L., Sporn, M.B. and Bissell, M.J. (1990) TGF-beta is a mediator of wound-related Rous sarcoma virus tumorigenesis. *Science*, **248:**1656-1660.

Singer, B. (1989) Structure-function relationships in alkylation damage and repair. In: *DNA Repair Mechanisms and Their Biological Implications in Mammalian Cells* (Lambert, M.W. and Laval, J., eds.), Plenum Press, NY, p1-17.

Singer, B., Chavez, F., Goodman, M.F., Essignmann, J.M. and Dosanjh, M.K. (1989) Effect of 3′ flanking neighbors on kinetics of pairing of dCTP or dTTP opposite $O^6$-methylguanine in a defined primed oligonucleotide when *Escherichia coli* DNA polymerase is used. *Proc. Natl. Acad. Sci., USA*, **86:**8271-8274.

Singer, B., Chavez, F., Spengler, S.J., Kusmierek, J.T., Mendelman, L. and Goodman, M.F. (1989) Comparison of polymerase insertion and extension kinetics of a series of $O^2$-alkyl deoxythymidine triphosphates and $O^4$-methyl deoxythymidine triphosphate. *Biochem.*, **28:**1478-1483.

Singer, B. and Dosanjh, M.K. (1990) Site-directed mutagenesis for quantitation of base-base interactions at defined sites. In: *Alkylation Damage, Repair and Replication* (Singer, B., ed.) *Mutation Res.*, in press.

Smith, C.L. and Cantor, C.R. (1989) Construction of physical maps and their application in finding genes. In: *Molecular Genetics of Neuromuscular Disease*. (Rowland, L.W., Wood, D.S., Schon, E.A. and DiMauro, S., eds.) Oxford Univ. Press, Oxford, p172.

Smith, D.L., Kleo, S.R. and Cantor, C.R. (1988) Pulsed field gel electrophoresis and the technology of large DNA molecules. In: *Genome Analysis: A Practical Approach* (Davies, Kay, ed.) IRL Press, Oxford, p41.

Smith, C.L., Yu, M.T. and Cantor, C.R. (1988) Phased approaches to mapping the physical structure of entire chromosomes. In: *Architecture of Eukaryotic Genes* (Kahl, G., ed.) CNG Verlagsgesellshaft, Weinheim, FRG, p489.

Sommers, C.L., Walker-Jones, K., Heckford, S.E., Worland, P., Valverius, E., Clark, R., McCormick, F., Stampfer, M., Abularach, S. and Gelmann, E. (1989) Vimentin rather than keratin expression in some hormone-independent breast cancer cell lines and in oncogene-transformed mammary epithelial cells. *Cancer Res.*, **49**:4258-4263.

Sorenson, S. Rubin, E., Pollster, H., Mohandas, N. and Schrier, S. (1990) The role of alpha globin chain accumulation in the pathophysiology of beta thalassemia. *Blood*, **75:**1333-1336.

Stoker, A.W., Hatier, C. and Bissell, M.J. (1990) The embryonic environment strongly attenuates *v-src* oncogenesis in mesenchymal and epithelial tissues, but not in endothelia. *J. Cell. Biol.*, **111**:217-228.

Stoker, A.W. and Sieweke, M.H. *V-src* induces clonal sarcomas and rapid metastasis following transduction with a replication-defective retrovirus. *Proc. Natl. Acad. Sci., USA,* in press.

Streuli, C.H. and Bissell, M.J. (1990) Expression of extracellular matrix components is regulated by substratum. *J. Cell. Biol.*, **110(4):**1405-1415.

Steuli, C.H. and Bissell, M.J. (1990) Mammary epithelial cells, extracellular matrix and gene expression. In: *Breast Cancer: Cellular & Molecular Biology 2* (Lippman, M.E. and Dickson, R., eds.) Kluwer Academic Publs., Norwell, MA, in press.

Stubbs, J.D., Lekutis, C., Zetti, K., But, A., Yuzuki, D., Srinivasan, U. and Parry, G. (1990) cDNA cloning of a mouse mammary epithelial cell surface glycoprotein reveals the existence of EGF-like domains linked to factor VIII-like sequences. *Proc. Natl. Acad. Sci., USA,* in press.

Talhouk, R., Streuli, C., Barcellos-Hoff, M.H. and Bissell, M.J. The extracellular matrix. In: *Fundamentals of Medicine Biology* (Bittar, E., ed.) PAI Press, in press.

Taylor-Papadimitriou, J., Stampfer, M., Bartek, J., Lewis, A., Boshell, M., Lane, E.B. and Leigh, I.M. (1989) Keratin expression in human mammary epithelial cells cultured from normal and malignant tissue: Relation to *in vivo* phenotypes and influence of medium. *J. Cell Sci.*, **94**:403-413.

Tenforde, T.S., Kavanau, K.S., Afzal, S.M.J. and Curtis, S.B. (1990) Host cell cytotoxicity cellular repopulation dynamics, and phase-specific cell survival in x-irradiated rat *Rhabdomyosarcoma* tumors. *Radiat. Res.* **123**:32-43.

Tenforde, T.S., Montoya, V.J., Afzal, S.M.J., Parr, S.S. and Curtis, S.B. (1989) Response of rat *Rhabdomyosarcoma* tumors to split doses of mixed high- and low-LET radiation. *Int. J. Radiat. Oncol. Biol. Phys.* **16**:1529-1536.

Valverius, E., Bates, S.E., Stampfer, M., Clark, R., McCormick, F., Salomon, D.S., Lippman, M.E. and Dickson, R.B. (1989) Transforming growth factor alpha and its receptor in human oncogenic transformation. *Mol. Endocr.*, **3**:203-214.

Valverius, E., Walker-Jones, D., Bates, S.E., Stampfer, M.R., Clark, R., McCormick, F., Dickson, R.B. and Lippman, M.E. (1989) Production of and responsiveness to transforming growth factor β in normal and oncogene transformed human mammary epithelial cells. *Cancer Res.*, **49**:6269-6274.

Van Houten, B., Gamper, H., Hearst, J.E. and Sancar, A. (1988) Factors influencing the assembly and turnover of ABC exonuclease. In: *Mechanisms and Consequences of DNA Damage Processing (Friedberg, E. and Hanawalt, P., eds.) Alan R. Liss, NY, p79-86.*

Voutilainen, P., Widness, J.A., Clemons, G.K., Schwarz, R. and Teramo, K. (1989) Amniotic fluid erythropoietin predicts fetal distress in Rh-immunized pregnancies. *Am. J. Obstet. Gynocol.*, **160**:429-434.

Walen, K.H. and Stampfer, M.R. (1989) Chromosome analyses of human mammary epithelial cells (HMEC) at stages of chemically-induced transformation progression to immortality. *Cancer Genet. Cytogen*, **37**:249-261.

Walian, P.J. and Jap, B.K. Three-dimensional electron diffraction of *PhoE* Porin to 2.8Å resolution. *J. Mol. Biol.*, in press.

Walker-Jones, D., Valverius, E.M., Stampfer, M.R., Lippman and Dickson, R.B. (1989) Transforming growth factor beta (TGFβ) stimulates epithelial membrane antigen expression in normal and oncogene-transformed human mammary epithelial cells. *Cancer Res.*, **49**:6407-6411.

Warkentin, T.E, Barr, R.D., Ali, M.A., and Mohandas, N. (1990) Recurrent acute splenic sequestration crisis due to interacting genetic defects: Hemoglobin SC disease and hereditary spherocytosis. *Blood*, **75(1)**:266-270.

Whaley, W.L., Michaels, F., MacDonald, M.E., Romano, D., Zimmer, M., Smith, B., Leavitt, M.B., Haines, J.L., Gilliam, T.C., Zehetner, G., Smith, C., Cantor, C.R., Fischauf, A.-M., Wasmuth, J.J., Lehrach, H. and Gusella, J.F. (1988) Mapping of D4S98/S113 confines the Huntington's defect to a reduced physical region at the telomere of chromosome 4. *Nucl. Acids Res.*, **16**:117-169.

Widness, J.A.; Philipps, A.F., and Clemons, G.K. (1990) Erythropoietin levels and erythropoiesis at birth in infants with Potter Syndrome. *J. of Pediatrics*, **117**:155-158.

Widness, J.A.,. Sanengen, T., and Haga, P., Clemons, G.K., Myhre, K. and Halvorsen, S. (1989) Correlation of plasma erythropoiesis stimulating factor(s) and immunoreactive erythropoietin levels during rapid growth in the mouse. *Acta Phys. Scand.*, **136**(4):527-533.

Wong, M., Schimmerling, W., Phillips, M.H., Ludewigt, B.A., Landis, D.A., Walton, J.T. and Curtis, S.B. (1990) The multiple Coulomb scattering of very heavy charged particles. *Med. Phys.* **17(2)**:163-171.

Worgul, B.V., Medvedovsky, C., Powers-Risius, P. and Alpen, E.L. (1989) Accelerated heavy ions and the lens; IV: Biomicroscopic and cytopathological analyses of the lenses of mice irradiated with 600 MeV/amu iron-56 ions. *Radiat. Res.*, **120:**280-293.

Yang, T.C., Craise, L.M., Mei, M. and Tobias, C.A. (1989) Neoplastic cell transformation by high-LET radiation: molecular mechanisms. *Adv. Space Res.*, **9(10):**131-140.

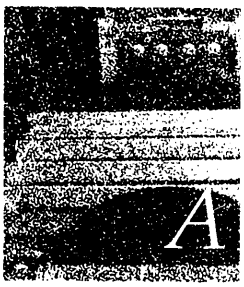
Yang, T.C., Grunenert, D.C., Holley, W.R. and Curtis, S.B. (1990) Response of cultured human airway epithelial cells to x-rays and energetic $\alpha$-particles. *Int. J. Radiat. Biol.*, in press.

Yang, T. and Nachtwey, D.S. (1990) Radiological health risks for exploratory class missions in space. In: *Symp. of 8th IAA Man in Space*, in press.

Yang, T.C., Stampfer, M.R. and Tobias, C.A. (1989) Radiation studies on sensitivity and repair of human mammary epithelial cells. *Int. J. Radiat. Res.*, **56:**605-609.

Yang, T.C. and Tobias, C.A. (1989) Molecular lesions important for neoplastic cell transformation of mouse (C3H10t/2) and human epithelial cells by ionizing radiation. In: *Cell Transformation Systems Relevant to Radiation-Induced Cancer in Man* (Cheedwick, K., ed.) IOP Publisher Ltd., p301-308.

Zimmerman, R.J., Chan, A. and Leadon, S.A. (1989) Recombinant human tumor necrosis factor causes damage in murine tumor cells treated *in vitro*. *Cancer Res.*, **49:**1644-1648.



# RESEARCH MEDICINE AND RADIATION BIOPHYSICS

*The long tradition of the Research Medicine and Radiation Biophysics Division is founded on the application of advanced technology to the service of medicine.*

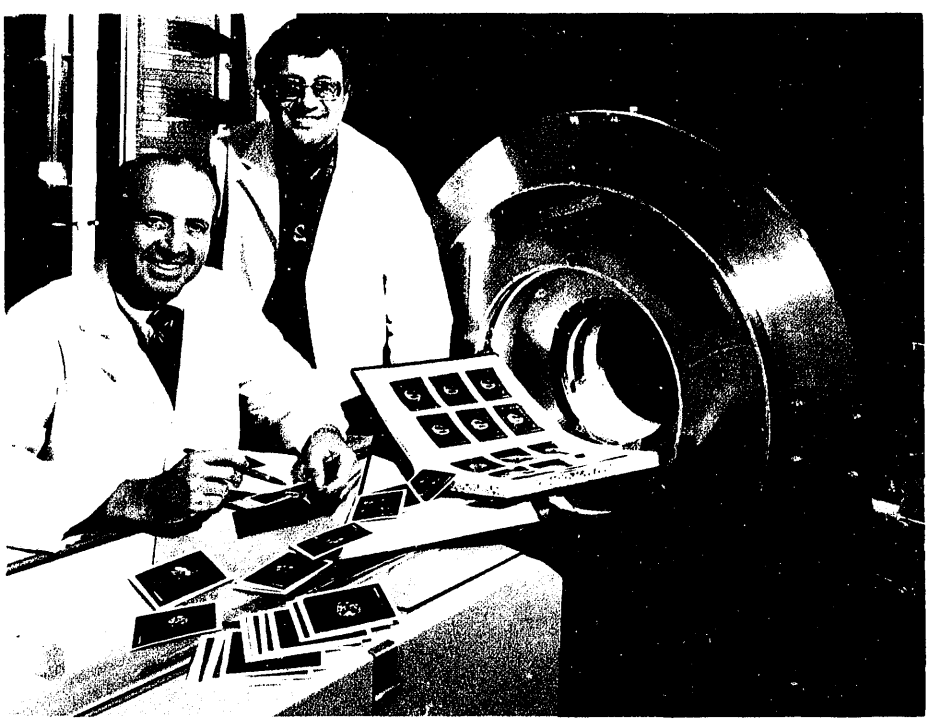
A DIRECT LINE CAN BE DRAWN BETWEEN THE EARLIEST biomedical experiments at the Radiation Laboratory on the Berkeley campus and the work now under way in the Research Medicine and Radiation Biophysics Division. Though 50 years of intellectual and technological evolution have led to new research directions, the focus of the Division continues to be the investigation of human physiology and disease, using the tools of nuclear physics. The early use of radionuclides as therapeutic agents is reflected today in their application as tracers used in conjunction with advanced diagnostic imaging systems; the earliest use of cyclotron-produced particles for cancer therapy finds its parallel in ongoing cancer therapy trials with heavy ions at LBL's Bevalac accelerator. In addition, a major effort is now under way to understand the genetic risk factors at work in atherosclerosis—a logical extension, in fact, of the first Donner Laboratory studies of lipoproteins and heart disease more than 40 years ago.

In looking to the future, we can anticipate continuing evolution and growth in each of these areas. In addition, the first steps have been taken in a wholly new direction: the exploitation of coherent soft x-rays from the Advanced Light Source (ALS) for a new generation of imaging experiments in the life sciences. A proposed Life Sciences Center would provide synchrotron radiation beamlines and experimental facilities for protein crystallography, x-ray microimaging and microholography, and x-ray spectroscopy. The Center would also provide a full complement of laboratory facilities for local and visiting scientists. The ALS will thus serve a well-established community of crystallographers with high-energy x-rays, but its most exciting attribute is the laserlike synchrotron light it will produce in the ultraviolet and soft x-ray regions of the spectrum. At these longer wavelengths, the coherent properties of the light offer us the unprecedented opportunity to look into living cells at resolutions approaching 10 billionths of a meter.

*Radioisotopes were first used to treat human disease by John Lawrence (top) brother of LBL founder Ernest O. Lawrence (bottom), who received the Nobel Prize in 1939 for inventing the cyclotron.*

## STUDIES OF BRAIN FUNCTION AND DISEASE

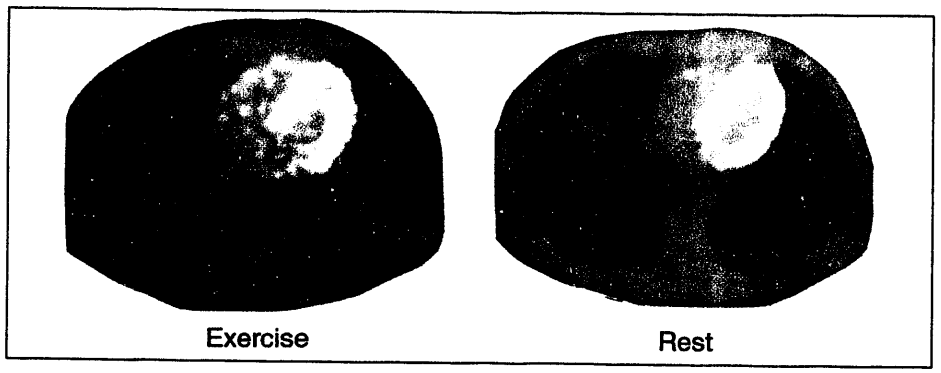
*The long tradition of the Research Medicine and Radiation Biophysics Division is founded on the application of advanced technology to the service of medicine. This convergence of technological development and medical research is especially evident in our broad program of research on brain and heart function and disease. In particular, our efforts focus on the use of positron-emission tomography (PET) and nuclear magnetic resonance (NMR, sometimes called magnetic resonance imaging or MRI). The PET program centers on the continuing use of the Donner 600-Crystal Tomograph, the world's highest-resolution instrument, for studies of various physiological processes. In several of the physiological studies, NMR serves as a complementary imaging technique. In addition, we continue to exploit classical radiotracer techniques in metabolic studies of schizophrenia, emotional disorders, and other neurological diseases.*



*RMRB Division Director Thomas Budinger (left) and Peter Valk study high-resolution brain images produced at the Donner 600-Crystal Positron Emission Tomograph, the world's highest resolution instrument for positron-emission tomography.*

### PET and NMR Studies

Positron-emission tomography is based on the use of metabolic tracers that contain a radioactive isotope that emits positrons (the electron's antiparticle). Each positron emitted by the isotope immediately annihilates with a nearby electron to produce two photons (particles of light). The simultaneous detection of these photons, traveling in opposite directions, pinpoints the position of the annihilation. Different tracers can be chosen, or synthesized, to investigate different kinds of metabolic activity, and time-depen–




*PET scan of normal heart shows how heart muscle absorbs rubidium-82 during and after exercise.*

dent studies can be carried out to assess the rates of metabolic processes. Many patient studies, for example, are done with fluorine-18-labeled fluorodeoxyglucose (FDG), an analog of the sugar glucose; like glucose, the analog is taken up by actively metabolizing tissue. The highest-resolution instrument now available for such studies is the Donner 600-Crystal Positron-Emission Tomograph, commissioned in 1986 for studies of brain metabolism. The resolution of this system is at least twice as good as that of any other instrument.

One particularly active area of research involving PET imaging has been the study of cerebral blood flow in Alzheimer's disease. Recent work has exploited a newly developed radiopharmaceutical labeled with iodine-122. This drug has a short half-life (210 seconds) that makes repeated measurements possible during one patient sitting. Thus, we can measure unstimulated blood flow values, then, following some stimulus, record the stimulated flow.

One such stimulus we have studied is $CO_2$ inhalation, which typically causes a significant increase in cerebral blood flow. Sixteen healthy adults, ranging in age from 27 to 78, and five Alzheimer's patients were subjected to PET scans before and after breathing $CO_2$ for 10 to 15 minutes. This physiological testing procedure is safe and well accepted, but these studies were the first ever done with PET. All control subjects showed consistent baseline patterns of cerebral blood flow, which then increased after $CO_2$ inhalation. This vascular system "reactivity," however, was less pronounced in the older subjects than in the younger ones. In the Alzheimer's patients, baseline blood flow rates were reduced in the temporal cortex (the lobes of the brain associated with hearing); however, vascular reactivity did not differ from that of the control subjects. Aging thus appears to diminish the ability of the cerebral vascular system to respond to challenge, but this impairment is not heightened by Alzheimer's disease. The abnormal blood flow patterns seen in Alzheimer's patients are probably not a cause of the disease, but rather a result of diminished glucose utilization.

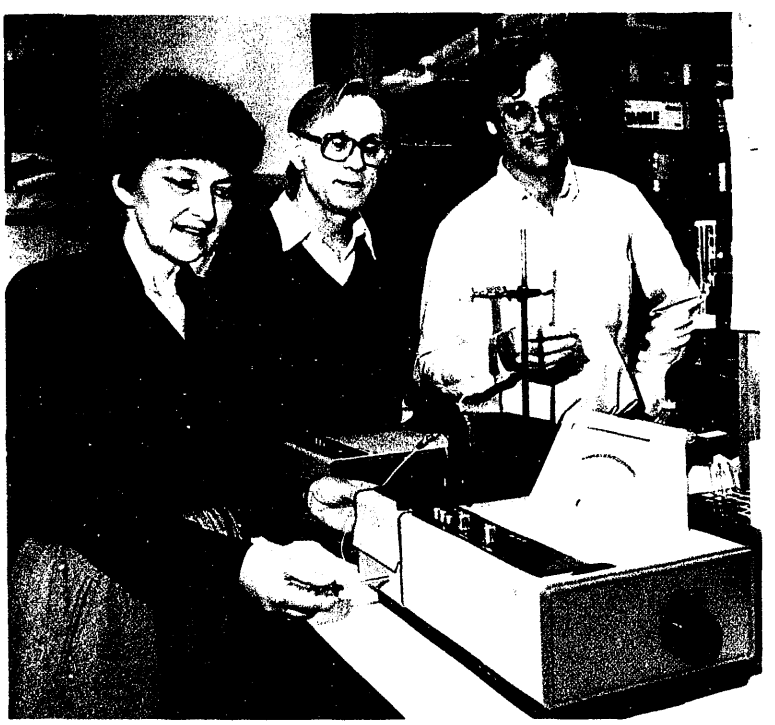
In other studies, PET and NMR are used in concert to reveal changes in brain anatomy and physiology. Whereas PET is a probe of metabolism, normal proton-NMR yields high-resolution anatomical maps based on variations in the distribution and tissue environment of water in the body. In one investigation, the two techniques were used to follow changes in brain tissue in patients exposed to heavy-ion radiation for treatment of arteriovenous malformations (abnormal clusters of blood vessels that are prone to bleeding). An understanding of such changes is important in the context of

*Chet Mathis injects a solution of a precursor compound into a tube that runs into a glovebox where radioactive labeling will occur to form a PET tracer to study heart or brain disease.*

the heavy-ion therapy programs described on page 45. Together, the use of PET and NMR have allowed us to follow changes in glucose metabolism in the brain, changes in the permeability of the blood-brain barrier, and brain tissue edema and abnormal extracellular fluid characteristics that arise following radiation damage.

### Tracer Studies of Schizophrenia

A third type of study relies on neither PET nor NMR, but uses instead classical tracer techniques. In our work on schizophrenia and emotional disorders, for example, we looked at metabolism of methyl ($-CH_3$) carbon by injecting a carbon-11 or carbon-14-labeled substance, then measuring the activity of expired $CO_2$. Our findings showed that unmedicated schizophrenics metabolize methyl carbon much less efficiently than do normal patients, suggesting that they may suffer an enzyme deficit in the pathway for metabolism of methyl carbon. This could affect the activity of neurotransmitters, as well as other physiological processes. To pursue this investigation further, we plan to search the sequence of methyl-metabolism products for an enzymatic "bottleneck."



*RMRB researchers Tasha Kusubov, Tony Sargent, and Scott Taylor have conducted tests which show that schizophrenics metabolize methyl carbon much less efficiently than do normal patients. They next plan to try to determine where the metabolic process is being blocked.*

To detect extremely low levels of tracer, we have proposed a new type of mass spectrometer, in conjunction with the Nuclear Science and Physics Divisions. This "cyclotrino" would detect individual carbon-14 atoms, thus enhancing detection sensitivity by a thousand. Such an instrument would also have utility in studies of other metabolic diseases, of which more than 3,000 are known, and in studies of the metabolism of drugs and toxic agents.

### PET Imaging of Vascular Lesions

The 600-Crystal Tomograph, designed for brain scans of human subjects, can also be used for whole-body imaging of small animals. We have thus been able to undertake high-resolution studies to detect vascular lesions in large blood vessels of rabbits. To do this, blood platelets were labeled with gallium-68. The labeled platelets were then injected intravenously into rabbits in which a major artery had been scraped by a balloon catheter. The scraping was done to simulate disease-induced arterial lesions. Platelet

localization at the site of injury could be clearly seen in the high-resolution PET images, suggesting the possible use of the 600-Crystal Tomograph and gallium-labeled platelets for imaging vascular lesions in the carotid arteries of human subjects. Efforts are also underway to determine whether radiation-induced damage to smaller vessels might be detected with radiolabeled substances.

## MEDICAL IMAGING TECHNOLOGY

*Over the past decade, technological improvements have elevated NMR and PET imaging to the level of routine procedures in the diagnosis of many diseases. Continuing research, however, points toward even wider applications for these techniques and, especially, toward higher image resolution. For example, our NMR group has completed theoretical studies that provide a solid foundation for eventually conducting NMR with high magnetic fields, which promises higher resolution and higher signal-to-noise ratios in diagnostic images. The key to better PET images, on the other hand, is smaller detector crystals, which, because of their size, are better able to localize the source of a detected pair of photons. One requirement of smaller detectors is a more suitable scintillator than the bismuth germanate currently used. We recently described a promising candidate material, lead carbonate. We also synthesized a new series of benzamides as tracers for PET studies.*

### NMR Methods

Research applications of NMR spectroscopy in living organisms are wide ranging, including studies of aging, atherosclerosis, heart disease, and cancer. Many such investigations, however, are limited by the signal-to-noise ratios and by the image resolution that can be achieved with techniques currently available.

The way NMR imaging works is that certain nuclei absorb radio waves of characteristic frequency in a magnetic field. These "resonances" provide signals that are used to construct an image. Improvements in resolution are possible by operating at higher magnetic fields, but higher fields typically require the application of radio-frequency (rf) pulses of impractically high peak power. This is a consequence of the usual way of conducting NMR experiments: rf pulses of short duration (about 10 millionths of a second) are applied to the subject, followed by long relaxation times (about 1 second) during which data are collected.

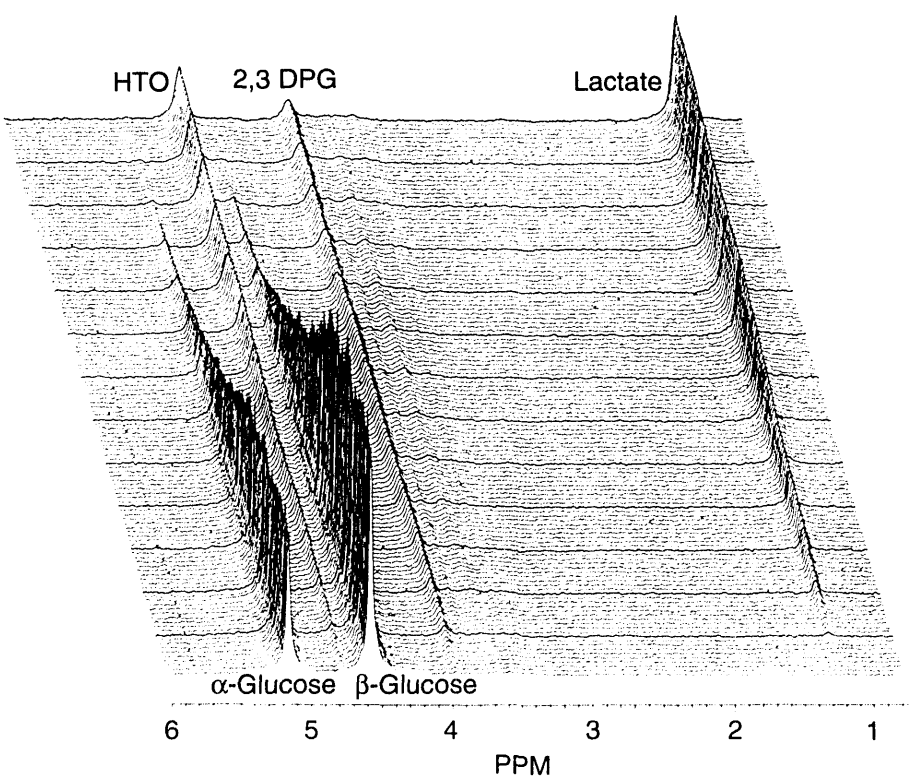
We have recently confirmed the feasibility of a technique known as stochastic excitation, which circumvents the high-peak-power requirements of conventional pulsed NMR. Stochastic NMR uses a noiselike rf excitation signal that is on almost continuously. The result is a reduction by several orders of magnitude in the peak-power requirement.

### Tritium NMR Studies of Metabolism

During the past year, we have utilized tritium NMR to study dynamic changes in cellular metabolism. A heavy isotope of hydrogen, tritium provides two unique advantages as a label for detection with NMR spectroscopy: It has the greatest NMR sensitivity of any nucleus and no background signal. Using tritium-labeled glucose as a tracer in mammalian red blood cells, we can observe metabolites from glycolysis and the pentose shunt—two different metabolic pathways by which sugar is broken down in the body.

To study the metabolism of deoxyglucose, an important compound used as a tracer in PET studies, we used tritium-labeled glucose and dexoyglucose. The experiments demonstrated that deoxyglucose is metabolized via the pentose shunt under anaerobic conditions and that the onset of this metabolism is correlated with the initial glucose concentration. These findings may help explain anomolous PET studies of diseased cardiac tissue.

In an extension of this work, we have used cardiac muscle cells from the newborn rat as a model to study metabolism in cardiac tissue. Taurine is an amino acid found at high concentrations in the heart. We examined the effect of taurine on glycolysis under anaerobic conditions (which occur in the body when cardiac cells are deprived of blood flow, as during a heart attack). Using tritium-labeled glucose, we observed that the rate of glycolysis doubled in the presence of taurine. It thus appears that at times of




*This stacked plot of NMR spectra obtained at 30-minute intervals shows consumption of tritiated glucose by red blood cells. Two forms of glucose (α and β) present in early spectra (bottom of figure) disappear as the sugar is broken down by glycolysis. Intermediate and final products (HTO; 2,3 DPG; lactate) of glycolysis predominate in the late spectra (top of figure).*

oxygen deprivation, taurine helps to keep cardiac cells viable. Studies are presently underway to determine whether the effect on glycolysis is due to a metabolic or an osmotic effect. This work represents the first time NMR has been used to study metabolism of heart tissue in culture.

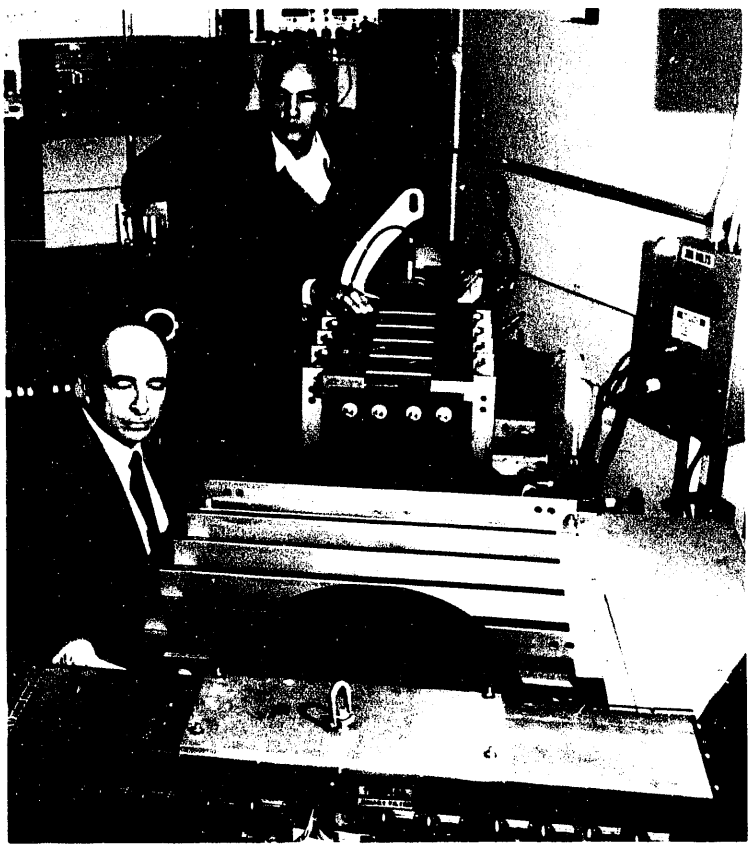
### Developments in PET Technology

Two especially notable developments have characterized our recent efforts to improve the state of current PET technology. The first was the finding that lead carbonate ($PbCO_3$) is an effective scintillator—that is, a substance that glows when struck by radiation. In a positron-emission tomograph, scintillators absorb the x-ray photons emitted during positron annihilation and emit, in turn, visible photons to be detected by a standard phototube. These scintillators embody three critical properties: high density, good light output, and a short fluorescent decay lifetime (which enables high photon count rates to be handled). Because lead carbonate appears promising on all three counts, three U.S. commercial firms have begun research on developing optical-quality crystals for use in PET devices, as well as in detectors for high-energy physics.

The second development is the synthesis of a new series of fluorine-containing benzamides (a class of benzene-ring molecules) as tracers for PET studies. These compounds have high affinity, as well as selectivity, for certain nerve-cell receptors that bind dopamine, a neurotransmitter. Studies using these compounds may have broad clinical relevance: Changes in nerve-cell receptor populations have been implicated in schizophrenia, Parkinson's disease, and many other neurological disorders.

## RADIOTHERAPY AND RADIOSURGERY WITH HEAVY IONS

*In distinct contrast to x-rays, heavy ions and neutrons are especially effective in killing oxygen-deficient cancer cells. In addition, ions can penetrate deeply into the body, then deposit their energy abruptly at the end of their path. Furthermore, this so-called Bragg peak of delivered dose can be modulated in three dimensions, so that a well-defined volume of tissue is irradiated. As a consequence, the radiotherapy program at LBL's Bevalac accelerator has been able to demonstrate unequivocally that superior local cancer control can be achieved with heavy ions for a number of different tumors. Our programs in this area are proceeding on course, with a central aim being a continuing evaluation of different ion species with respect to their effectiveness in treating different types of cancer. Nonclinical research proceeds in parallel, aimed mainly at understanding the physical and biological characteristics of heavy-ion beams and their effects in the brain.*



*Physician Joseph Castro (front) and Jose Alonso check adjustments on an LBL-developed beam guidance system used to deliver particle beams for radiotherapy at the Bevalac accelerator.*

## Radiotherapy

Radiotherapy used alone or in combination with surgery is an effective treatment for many forms of human cancer. However, in approximately 25 to 35 percent of patients, conventional radiotherapy techniques are not sufficient for control of resistant tumors, or tumors adjacent to critical normal tissues. At Lawrence Berkeley Laboratory, studies with heavy charged particles have been ongoing since the mid 1970s. We have now treated over 1200 patients with helium and neon ion beams as part of a clinical research program supported by the Department of Energy and the National Cancer Institute.

For such tumors as chordoma or chondrosarcoma at the base of the skull or adjacent to the spinal cord, the localized deposition of radiation inherent in charged-particle therapy has resulted in approximate doubling of the rate of local tumor control over that achievable with x-ray therapy (70 percent versus 35 percent), with follow-up ranging from one to 11 years. Even more favorable results have been observed in irradiation of small melanomatous tumors in the eye: Tumor control has been achieved in 97 percent of the over 300 patients treated. Eighty-five percent of the patients have retained their eyes and about half have kept useful post-treatment vision.

With heavier particles, such as neon ions, preliminary results in approximately 250 patients demonstrate promising local control rates for tumors of the salivary gland, prostate gland, and paranasal sinuses, as well as soft tissue and bone tumors. In addition to the advantage of localized deposition of radiation dose, heavier ions offer a greater biological effect on tumor cells. They are particularly promising for tumors with cellular characteristics that make conventional irradiation ineffective—that is, tumors that are composed of radioresistant oxygen-deficient cells or have a high capacity for repair of conventional radiation damage.

During the next year, we will continue studies comparing heavy charged particles such as neon to x-ray irradiation and helium-ion therapy. We also will continue development of a new beam delivery system, called "dynamic conformal-beam scanning," which will more precisely localize the high-dose radiation zone to the three-dimensional shape of a tumor and therefore spare an even greater amount of critical normal tissue. Physical, biological, and clinical studies will be implemented over the next three years to bring this exciting form of beam delivery into daily clinical use. Our goal in this therapy is to reduce the national standard for radiotherapy local-treatment failures from 30 percent to less than 10 percent. Combined with

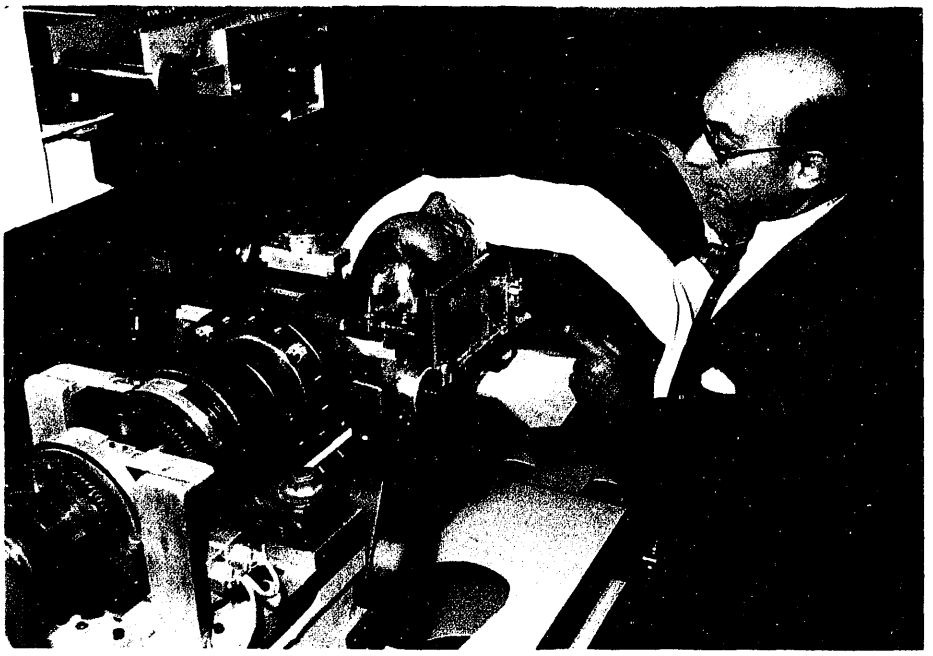
multimodality therapy, such as surgery and chemotherapy, this should lead to greater survival rates and improved quality of life for cancer patients.

### Radiosurgery

Our stereotactic radiosurgery clinical-research program continues its studies on the use of helium ions to treat life-threatening intracranial disorders called arteriovenous malformations (AVMs). An AVM is a pathological cluster of blood vessels that are prone to hemorrhaging; AVMs in the brain are particularly dangerous. The ultimate goal of our work on AVMs is the transfer of safe, reliable, and reproducible treatment strategies to facilities based at community hospitals. Accordingly, our program is designed to optimize the dose, treatment volume, ion type, beam delivery technique, and treatment schedule for irradiation. For several years, we have concentrated especially on deep-seated, surgically inaccessible intracranial AVMs.

At the end of 1990, the helium-ion radiosurgery program had treated over 300 patients with inoperable AVMs, and we were in the process of analyzing the conditions of all treated patients who had been followed for three years or more. In a group of more than 100 patients for whom follow-up test results were available, results showed complete or partial cure in all cases. In about 80 percent of patients, the AVM was completely obliterated.

We have demonstrated that with the heavy-ion radiosurgical method, the radiation dose to normal brain structures both adjacent to and remote from the AVM is relatively low, particularly when compared to x-ray and other conventional irradiation techniques; this difference appears especially marked in the treatment of larger AVMs. We consider the overall results with our patients to be favorable: While providing satisfactory protection of critical brain structures, the treatment is successfully obliterating a major-



*Physician Jacob Fabrikant checks the mask and frame that will hold a patient's head in place during irradiation of an arteriovenous malformation (AVM) in the brain. In about 80 percent of patients, this treatment completely obliterates the AVM.*

ity of life-threatening inoperable AVMs, including many more large, irregularly shaped lesions than appear to be amenable to other irradiation techniques.

We are also pursuing a more extensive neuroradiologic follow-up by using NMR and PET imaging to evaluate the time course of radiation-induced changes in AVMs and surrounding tissue. We are using several different PET tracers to assess changes in glucose metabolism, regional blood flow, and the integrity of the blood-brain barrier, and we are correlating these changes with NMR data.

### Basic Radiation Biophysics

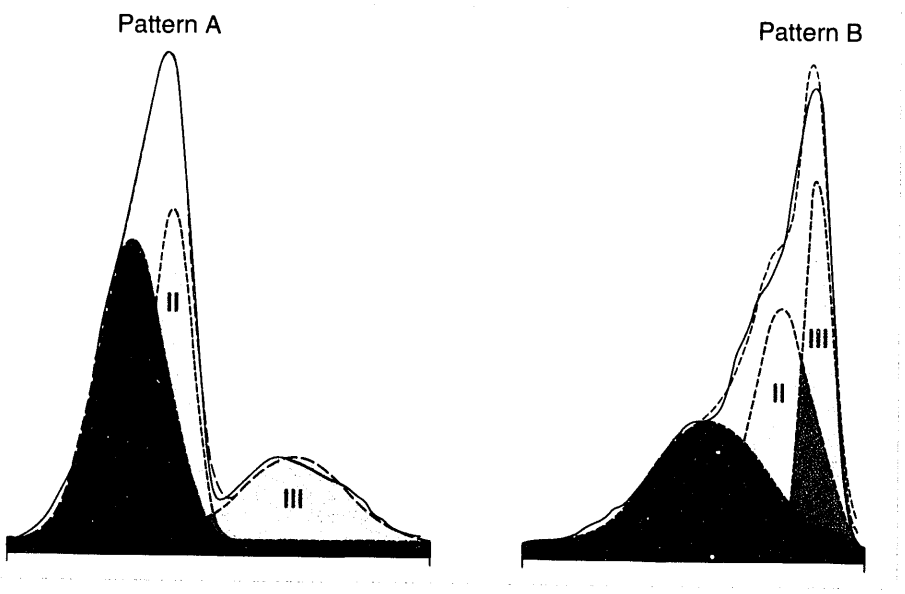
Underlying the radiotherapy and radiosurgery programs are continuing basic biophysical studies on the properties of the ion beams being used medically. (Pertinent work is also carried out in the Cell and Molecular Biology Division; see pages 2–5.) Prospects are encouraging that, as a result of these studies, a more accurate ion-dose calculation method will soon be available. We are also doing heavy-ion beam characterization and fragmentation studies, comparisons of dose distributions obtained with different forms of radiation and different irradiation geometries, studies of brain-cell DNA damage and repair, and studies in animal and human models of metabolic and physiologic changes induced by radiation.

## LIPOPROTEINS AND ATHEROSCLEROSIS

*A major program in the Research Medicine and Radiation Biophysics Division focuses on the structure, metabolism, and genetic determinants of lipoproteins—lipid/protein particles that transport cholesterol and other lipids (fat-related substances) in the bloodstream. The principal aim of the studies is to better understand the relationship between lipoproteins and the development of coronary artery disease. How do genetic and environmental factors affect lipid transport and the development of atherosclerosis? To find out, we are unraveling the processes that give rise to the major lipoprotein classes. The program uses a multidisciplinary approach—including human and molecular genetics, cell biology, metabolism, biophysics, statistics, and mathematics—to study atherosclerosis development.*

### Lipoprotein Classes

The major lipoprotein classes include high-density lipoproteins (HDL) and low-density lipoproteins (LDL). A primary focus during the past year has been investigation of pathways and mechanisms for the origins of both large and small HDL particles. Increased levels of large HDL particles in blood plasma are generally associated with increased plasma concentrations of HDL-cholesterol. In the U.S. population, high levels of HDL-cholesterol are, in turn, correlated with a decreased risk of coronary heart dis-




*LDL subclass pattern A shows relatively high levels of large, buoyant LDLs (I and II) and relatively low levels of small, dense LDLs (III). In pattern B, which is associated with increased risk of heart disease, the small, dense LDLs predominate.*

ease. Interest in small HDL particles is based principally on their likely role in initiating the transport of cholesterol from cells throughout the body to the liver for excretion, a process called "reverse cholesterol transport."

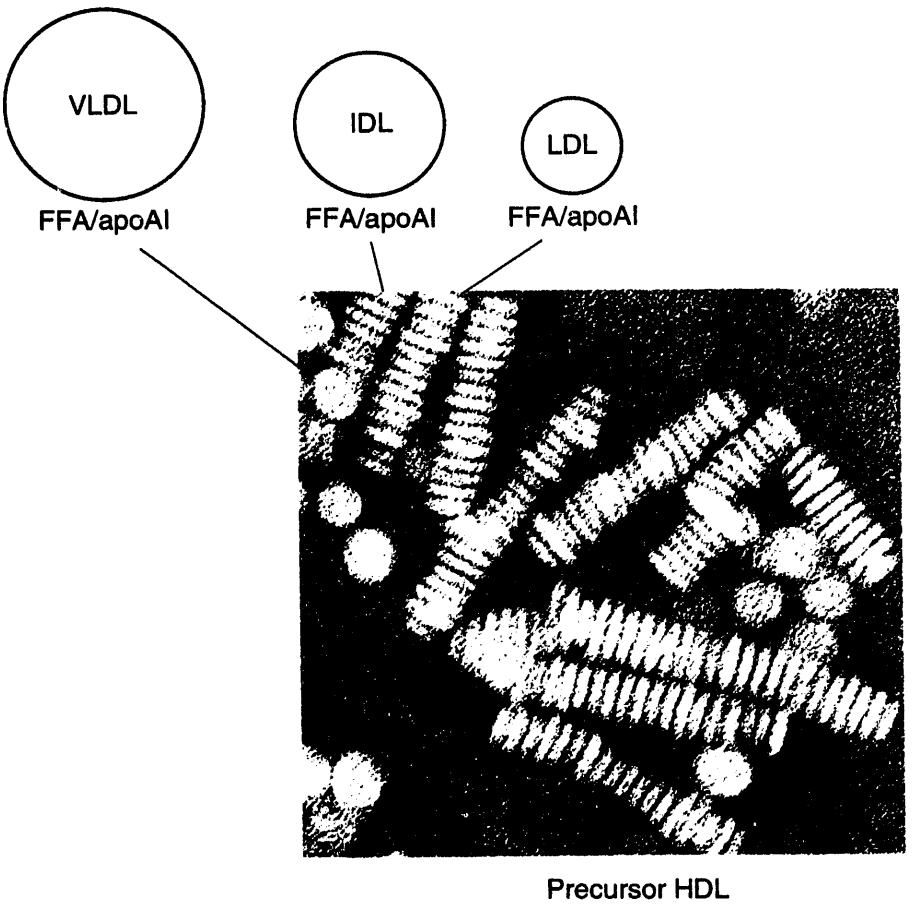
In humans, the liver is a major site of HDL synthesis. To better understand the synthesis and secretion of HDL by the liver, we are studying the metabolism of HDL secreted by a human liver-tumor–derived cell line. Our studies suggest that HDL secreted by these cells is an excellent model for elucidating the role of an enzyme called LCAT in lipoprotein metabolism. This enzyme, in conjunction with HDL, plays a key role in reverse cholesterol transport. To examine the role of specific HDL subclasses in reverse transport, we are testing the effects, on well-defined synthetic HDL analogs, of enzymes and other proteins that are known participants in cholesterol metabolism. Studies are also underway to test whether specific analogs containing apoAI (the primary protein component of HDL) can stimulate cholesterol release from lipid-laden cells.

Our research programs are also concerned with the low-density lipoproteins, several different subclasses of which have been identified. These subclasses are characterized by differences in size, density, and lipid and protein composition. Further, analyses of plasma LDL in large numbers of human subjects have revealed considerable variability in the distribution patterns of LDL subclasses. These patterns can be grouped into two distinct categories, which we have designated LDL subclass patterns A and B. Pattern A is found in 60 to 70 percent of normal subjects, pattern B in 30 to 40 percent; an intermediate pattern is observed in up to 10 percent of the population.

Pattern B, which is produced by a dominant gene, is linked to increased risk of premature cardiovascular disease. We have established that this trait is fully expressed in men after the age of about 20 and in women after menopause, indicating the importance of developmental and hormonal factors in the production of this lipoprotein profile. We also showed that this

*RMRB researcher Trudy Forte (left) and instructor Kim Snipes of Sierra College in Rocklin, California, examine a gel stained for lipoproteins. Forte has demonstrated the existence of lipoprotein dissociation complexes that may be involved in the removal of excess cholesterol from the bloodstream.*

*Electron microscope image of dissociation complexes formed by the incubation of VLDL, IDL, and LDL with free fatty acids (FFA) and apoAI. The complexes, which are precursors for HDL, illustrate quantized size transformation of lipoprotein particles.*




trait is strongly linked with reductions in plasma HDL levels and with increases in levels of intermediate-density lipoproteins (IDL) and lipoproteins rich in triglycerides (the major energy fats). These changes all tend to increase atherosclerotic risk and, for this reason, the newly discovered gene has been designated *ALP* (Atherogenic Lipoprotein Profile). The increased risk of coronary disease predicted for individuals carrying this gene has already been confirmed by a case-control study showing a two- to threefold increased heart-attack risk for individuals with LDL subclass pattern B.

The biochemical basis for the differing LDL subclass patterns is, as yet, not understood. One hypothesis suggests that pattern B is associated with an altered carbohydrate synthetic pathway in the liver, which may in turn be linked to variations in LDL metabolism in the liver. We tested this hypothesis and demonstrated that the carbohydrate content of LDL protein in pattern B is significantly reduced compared to that in pattern A. The carbohydrate content of LDL lipid/carbohydrate molecules was also reduced in pattern B. Reduction in carbohydrates may influence the subsequent metabolism of the LDL and may play a major role in determining the atherosclerotic risk associated with these particles.

LDL, as well as triglyceride-rich lipoproteins, may undergo "quantized" size transformations as a result of dissociation of proteins and lipids from the lipoprotein particles. Free fatty acids in plasma may promote such

changes in the presence of HDL or apoAI. We are presently investigating this possibility. In the past year, we have demonstrated quantized transformation during incubation of LDL, IDL, and very low-density lipoprotein (VLDL) with free fatty acids and apoAI. The resulting "dissociation complexes" appear as variously sized stacks of disks in electron micrographs. The complexes, which contain apoAI, as well as surface lipids dissociated from LDL (or IDL and VLDL), are precursors for HDL particles and may play an important role in the removal of excess cholesterol.

Other studies are likewise aimed at ultimately understanding the relationships of fatal coronary heart disease to lipoprotein subclasses and other established risk factors. One such study involves 1,961 men and 423 women who were employed at the Lawrence Livermore National Laboratory between 1954 and 1957. We intend to extend earlier investigations to 30 years of follow-up. Preliminary analyses suggest that strong relationships exist between lipoprotein subclass measurements and the subsequent development of heart disease and stroke.

### The Dairy Research Institute for Genetics and Nutrition

LBL's Dairy Research Institute for Genetics and Nutrition is a multidisciplinary research unit funded by the National Dairy Promotion and Research Board. In both human and animal studies, the Institute is testing new approaches to understanding and modifying the effects of dairy fat on heart disease.

In the past year, a major achievement at the Institute has been development of a very unique system to study the influence of apoAI on HDL structure, distribution, and metabolism and the role of HDL in decreasing the risk of cardiovascular disease: We have produced two independent transgenic mouse lines that contain the human apoAI gene. The mouse strain from which the transgenic lines were derived was selected because of its susceptibility to atherosclerosis in response to dietary fat and cholesterol. The transgenic mice produce twice the normal amount of apoAI, about 95 percent of which is the human protein. Moreover, the mice produce the two predominant forms of human HDL rather than the single type of HDL particle characteristic of mice. The results demonstrate a dominant role of apoAI in determining HDL structure. The transgenic mice are being used to test whether increases in HDL levels can alter susceptibility to development of diet-induced atherosclerosis; they are providing the first direct evidence in a living organism that ApoAI has an immediate role in preventing heart disease.

The Institute is conducting a second mouse genetic study with Jackson Laboratory in Bar Harbor, Maine. The aim is to investigate the influence of specific mouse genes on the relationship between diet composition and atherosclerosis. Identifying the mouse genes which alter dietary susceptibility may help us to understand the effect of similar genes in humans.

Human diet studies at the Institute are exploring the influence of the two genetic LDL subclass patterns on response of individuals to dairy fats. Do such fats have differing effects on susceptibility to heart disease in individuals with these genetic traits? The effects of high-dairy-fat and low-dairy-fat diets in large families will allow studies of heritability of heart

*Edward Rubin (shown) and Ronald Krauss have created the first transgenic mice that carry the gene for human apoAI, the main protein found in HDL. The mice are providing the first direct evidence in a living organism that apoAI has an immediate role in preventing heart disease.*

disease risk in offspring. The clinical portion of these studies will be conducted at the Cholesterol Research Center near Alta Bates Hospital in Berkeley.

A second project involving human subjects is investigating the genes that influence response to dairy fats. Using the DNA obtained from large families, genes are being analyzed to determine which ones may possibly influence the effects of dairy fats on lipoprotein production and metabolism. This work is being done in collaboration with Children's Hospital in Oakland, California.

## BIOELECTROMAGNETICS RESEARCH PROGRAM

*Our bioelectromagnetics research program focuses on the effects of magnetic fields and of radio-frequency and microwave radiation on molecular, cellular, and tissue systems. The ultimate aim is twofold: (1) to address health and safety issues related to technologies that rely on high-intensity magnetic fields or on microwave and rf electromagnetic fields, and (2) to explore possible biomedical applications of such fields.*

### Health Effects of Powerline-Frequency Magnetic Fields

Powerline-frequency (60-hertz) magnetic fields, also called extremely-low-frequency (ELF) magnetic fields, are receiving increasing attention as an environmental agent that may influence human health. Several epidemiological studies have suggested that human exposure to ELF electric and magnetic fields is linked to the development of cancer, particularly leukemia. At this time, the increased risk appears to be about 20 percent for all leukemias and about 45 percent for acute myeloid leukemia. Additional large-scale epidemiological studies are now in progress.

LBL has one of several laboratories in the country that are studying the basic interaction of electric and magnetic fields with cellular systems. Since ELF fields are not energetic enough to damage DNA directly, our goal has been to identify ELF-induced alterations in cell-membrane functions that are critical to cell proliferation and division. This means examining cellular communication processes, such as calcium transport across the cell membrane.

Binding of hormones or other messenger molecules to the cell membrane of a lymphocyte (a type of white blood cell) causes calcium to enter the cell. Following the rise in intracellular calcium concentration, DNA synthesis in the cell nucleus increases, and cellular proliferation and division can ensue.

We have recently tested the hypothesis that ELF magnetic fields can also increase calcium transport. The model system we used was the rat thymocyte (a T lymphocyte produced by the thymus), which undergoes an abrupt increase in calcium transport in response to Con A, a chemical that induces mitosis, or cell division. We discovered that when thymocytes in

culture were treated with ELF fields for only 60 minutes in the presence of Con A, the resultant increase in calcium transport exceeded by up to two-fold the increase induced by Con A alone. Interestingly, the ELF field alone had no effect. This means that in combination with Con A, an ELF field can act to enhance events linked to mitosis. Currently, we are characterizing this cellular response to identify thresholds for field intensities and for duration of field exposure.

If this finding is generalizable and ELF fields can act as coinducers of mitosis for lymphocytes in the body, there might be therapeutic applications for immunosuppressed patients and for geriatric patients with reduced immune function. The coinduction response may also be relevant to the development of cancer, since overstimulation of mitosis for a prolonged period could lead to events associated with uncontrolled cell proliferation.

### Use of Liposomes and Microwave Fields for Drug Delivery

We have found that liposomes—microscopic, membrane-bound vesicles made in the laboratory—can be used to deliver therapeutic drugs on demand. Liposomes are made by mixing phospholipids (fat-related substances containing phosphorus) dissolved in an organic solvent with a drug dissolved in water. Vesicles form spontaneously, with a substantial portion of the drug trapped inside.

In animal studies, we have shown that a microwave field can be used to release the antibiotic drug Gentamicin from single or multiple liposome injection sites, or "depots," in fat or muscle tissue. A single depot can act as a reservoir for drug release with successive microwave treatments: When a single depot was exposed to a microwave field at 24-hour intervals, Gentamicin was released into the bloodstream with each exposure. Treatment of multiple depots with microwave fields resulted in additive release of Gentamicin into the bloodstream.

The maximal effective dose of a drug can vary widely during the day and night, according to the phase of the organism's biological clock. Thus, pulsative, on-demand, drug delivery, such as that provided by the microwave/liposome technique, is the best way to deliver many drugs.

In tissue-culture studies, we have shown that microinjection of drugs into target cells can be achieved using microwave fields and liposomes. After the cells have been coated with liposomes containing a drug of interest, a microwave field is applied to trigger release of the drug directly into the cells. We have accomplished this with various cell types—including lymphocytes, macrophages, and several tumor cell lines.

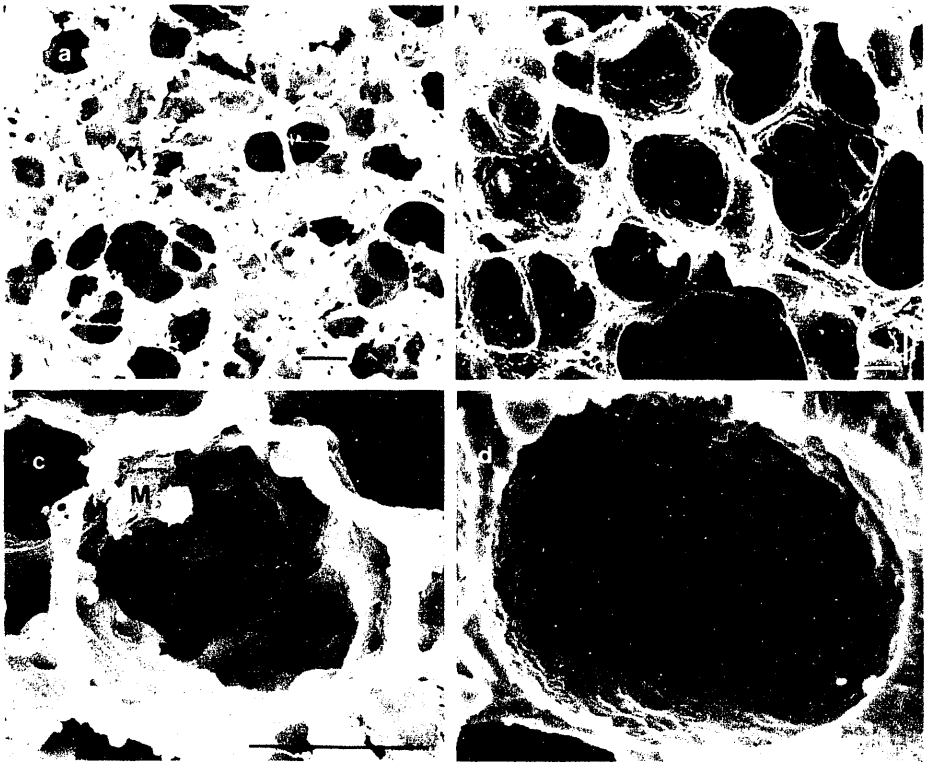
When injected into a patient, liposomes with antibody molecules attached would bind to specific target cells (such as cancer cells). Once this binding occurred, microwaves could be used to microinject the drug. This approach would reduce the total amount of drug needed to treat the patient—which would be especially desirable in the case of toxic drugs and those with serious side effects.

*Scientist Robert Liburdy and graduate student Cameron Sheeler check a microwave exposure device used to release therapeutic drugs from liposome vesicles.*

## LUNG MICROIMAGING

*The lung microimaging group is developing new electron microscope imaging techniques and applying them to clinically important questions on the structure and function of the lung. We have developed a low-temperature scanning electron microscope and are using it to study respiratory distress syndrome, asthma, and the effects of cigarette smoke on the lung.*

Neonatal respiratory distress syndrome affects between 40 and 50 thousand premature babies per year in the United States. The lungs of these babies, which have not matured, do not make the detergent-like surfactant that normally coats the inside of adult, child, and mature infant lungs and keeps the millions of tiny air sacs inflated. Without this surfactant, the lung collapses and the premature babies develop life-threatening difficulty in breathing. We are working to prove that surfactant floats on a thin, continuous foundation of water. Physiologists who model the lung have hypothesized that such a water layer exists as millions of soap bubbles, but this aqueous layer could not be demonstrated with early electron microscopes because such microscopes required samples to be dried prior to viewing. Our low-temperature scanning electron microscope allows us to look at the aqueous layer by keeping water in the lung preserved as ice. We have developed techniques to prepare lung tissue for this microscope, and we are now measuring the thickness, continuity, and extent of the aqueous lining of the lung. Such studies will be important in improving




*Frozen, hydrated lungs photographed at -190°C in the low-temperature scanning electron microscope (b and d) provide views of air-sac structure closer to that of the water-rich living state than do conventional dried samples (a and c) and allow us to study the characteristics of the liquid layer that lines the lung. Detergent-like surfactant floats on this liquid layer. Bar represents 20 microns (millionths of a meter).*

treatment and understanding the pathogenesis of respiratory distress syndrome.

We are also studying the amount and distribution of water in the airways of the lung and the role that this water plays in the origin and development of asthma. Millions of Americans suffer from asthma—periodic attacks of breathing difficulty due to obstruction of the flow of air in the airways. Inflammation, muscle contraction, and altered secretions contribute to this process; we are assessing the degree to which water causes the obstruction. By examining the effects of drugs and physiological mediators on airway structure in the hydrated state, we are working to better understand the controlling mechanisms of asthma.

Cigarette smoking has been shown to cause important chronic diseases of the lung: emphysema, chronic bronchitis, and cancer. We are concerned with the earliest effects of smoke on the lung and the lung's ability to defend itself against such inhaled irritants. The lungs might respond much as our eyes do when exposed to an irritant—by secreting a protective layer of liquid. We hypothesize that one of the first things cigarette smoke does to the lung is to cause an increase in the amount of water lining the air sacs. We are using the low-temperature scanning electron microscope to measure the amount of water in the lung at increasing intervals after exposure to cigarette smoke. We expect that this water represents one of the body's first attempts to counteract the deleterious effects of the many irritants present in smoke.

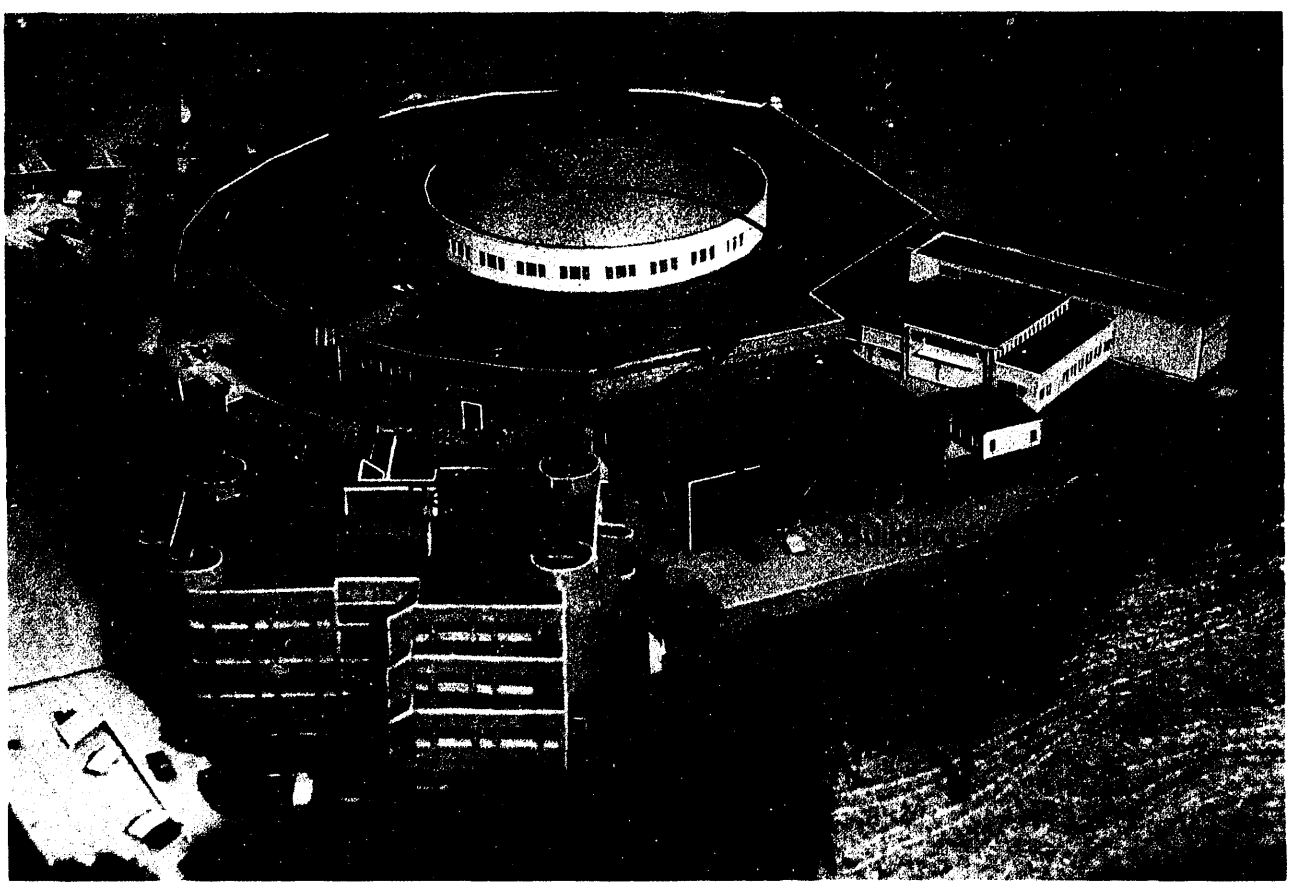
*Jacob Bastacky prepares to photograph a specimen of human lung. His studies of the aqueous lining of the lung are shedding light on respiratory distress syndrome, asthma, and the lung's response to cigarette smoke.*

In addition, we have been using the High-Voltage Electron Microscope at LBL's National Center for Electron Microscopy to visualize the constituents of the air-sac wall and to provide comparison data for the x-ray microscope images to be produced in future applications of the Advanced Light Source. We have also begun feasibility studies for a high-resolution, low-temperature scanning electron microscope to examine lung structure, and we have been developing apparatus for freezing lungs and other organs as rapidly as possible to produce specimens.

## ALS LIFE SCIENCES CENTER

*The Advanced Light Source (ALS), now under construction at LBL, is the first of a third generation of synchrotron light sources—facilities that produce intense radiation by constraining electrons to curved trajectories, forcing them as a consequence to give up energy in the form of light. By relying on special periodic magnetic arrays known as "wigglers" and "undulators," and by keeping its circulating electrons within the bounds of a narrow pencil-like beam, the ALS will produce soft x-rays and ultraviolet light of unprecedented intensity. For the life sciences, the ALS may open the door to high-resolution dynamic imaging of biological events and to practical microholography and x-ray microimaging, as well as providing a state-of-the-art facility for x-ray spectroscopy and crystallography. To exploit this resource for biology, we have therefore proposed an ALS Life Sciences Center, whose scope includes construction of an undulator beamline equipped for microimaging and microholography, a wiggler beamline equipped for spectroscopy and crystallography, and 11,100 square feet of fully equipped support laboratory and office space. The estimated cost of the project is $28.6 million; construction is scheduled to begin in 1992 and be completed in 1994.*

*Architectural model showing the ALS Life Sciences Center, where synchrotron light will be used to obtain unprecedented microscopic views of cells and their substructures. The Center will include two insertion devices (a wiggler and an undulator), together with their beamlines and experimental end stations, and 11,100 square feet of fully equipped laboratory and office space.*



## Background of the Proposal

The most powerful sources of x-ray and ultraviolet radiation (collectively, the XUV region of the electromagnetic spectrum) are synchrotron light sources, where electrons circulating in an accelerator at relativistic velocities emit light as a by-product of their motion. Missing so far has been XUV light with many of the useful properties that are associated with visible and infrared laser light.

In essence, the ALS, now under construction at the Lawrence Berkeley Laboratory, will provide such light to the experimental scientist. Though not a laser, the ALS will offer researchers laserlike radiation of unprecedented brightness at ultraviolet and soft x-ray photon energies. Thus, biologists can look forward to a host of new possibilities for the life sciences, including unprecedented microscopic views of cells and their substructures in a near natural state, insights into multimolecular aggregates of biological macromolecules, and even views of physical and chemical dynamics at high resolution. The ALS and other light sources of its generation are also certain to bring established methods of structural investigation, such as protein crystallography and x-ray spectroscopy, to new levels of technical achievement.

Toward these objectives, we have proposed a Life Sciences Center at the ALS. This Center will be a unique national resource for synchrotron-based research in the life sciences, providing a capability for entirely new lines of investigation. The Center will include next-generation synchrotron-based experimental facilities for x-ray microscopy, holography, spectroscopy, and crystallography, which will make possible the coordinated application of these techniques to studies in the life sciences. In addition, and most importantly, the Center will make it possible to bring forefront technical developments in physics and engineering to bear on basic and applied research problems in biology and medicine. The Center will also house crucial ancillary biological facilities necessary to make the ALS an effective resource for broadly based life sciences research.

As a basis for discussion within the life sciences community, a preliminary conceptual design proposal for the Life Sciences Center was prepared in 1988. The response to this proposal was a consensus that the Center should be part of a major structural biology facilities initiative proposed by the DOE's Office of Health and Environmental Research. In February 1990, a full conceptual design report for the Center was completed.

## Research at the Life Sciences Center

Because the wavelength of x-rays is far shorter than that of visible light, far smaller features can, in principle, be discerned: The sizes of the cellular features we hope to resolve at the ALS Life Sciences Center are roughly equivalent to the wavelength of the probing light. If this theoretical equivalence can indeed be approached in practice, using techniques currently available and others foreseen for the future, x-rays offer a profound opportunity to deepen our understanding of biological structure and structure-function relationships.

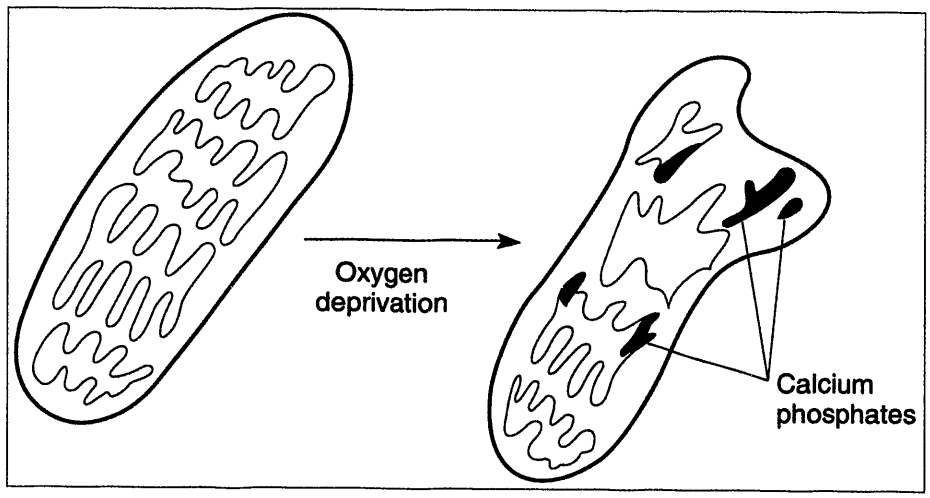
*An x-ray micrograph of a zymogen granule (a subcellular secretory structure) extracted from the pancreas of a rat. The image shows the general form of the granule in its watery environment, its nonuniform nature, and a feature in the lower right hand corner of the granule.*

In addition to the relationship between resolution and wavelength, x-rays offer many features that make them particularly valuable as probes of biological matter. Accordingly, the Center's scientific goals include (1) new applications of x-ray methods already established and widely used, such as protein crystallography; (2) full realization of the promise of newly emerging synchrotron-based microimaging methods, such as x-ray microscopy and holography; (3) assessing the feasibility of new technical approaches, such as merging spectroscopy and spatially resolved x-ray microimaging; and (4) the development of novel means of extending imaging resolution toward theoretical limits.

An important new scientific opportunity for the life sciences at the ALS will be afforded by the construction of x-ray microscopes and holographic imaging devices of a character and degree of development not heretofore possible. In conjunction with novel approaches for improved resolution, the promise of high-resolution microscopic x-ray imaging appears to be within reach. Even imaging techniques currently available at second-generation synchrotron radiation sources permit examination of biological material in a near natural state at resolutions never before achieved, and resolution may approach 10 nanometers (billionths of a meter) by the time the ALS is commissioned. Imaging with soft x-rays—x-rays with wavelengths between roughly 1 and 5 nanometers—can be "natural" because such radiation can penetrate much thicker objects than visible light, and thus some sectioning can be avoided. Also, the object can be viewed while suspended in water at atmospheric pressure and body temperature. Water is transparent to soft x-rays, whereas various elements normally present in biological material, particularly carbon and nitrogen, absorb these photons. This allows for contrast based on variations in the concentrations of natural substances, rather than by staining with foreign ones.

X-ray spectroscopy, which is based on absorption of x-rays of specific wavelengths by elements of biological interest, has an excellent record of achievement in several areas of biological, biochemical, and biophysical research, and the ALS will offer exciting new opportunities in this area. The extra capabilities that the ALS will bring to spectroscopic studies include the investigation of very dilute materials and the analysis of material that is spatially localized within some particular cellular region—a combination of microimaging and spectroscopy. Elemental mapping by x-ray spectroscopy will make it possible not only to measure quantitatively the presence of particular elements in a spatially resolved fashion, but also whether these elements are present in organic, or carbon-based, molecules: This method has the potential to provide direct information regarding the chemical form and reactive state of specific molecules within the cell.

The ALS will provide photons in two energy regions that will be at the forefront of innovative biological spectroscopy. The first region is that from 2 to 4 keV (thousand electron volts), which contains absorption peaks for the elements phosphorus, sulfur, chlorine, potassium, and calcium, all of great importance and interest in biology. The second energy region of particular interest is between 0.3 and 1.2 keV, where one finds absorption peaks for the first series of transition elements (scandium through zinc), almost all of which are biologically important, as well as the peaks of important light elements. It is in this range that the undulators planned for




*Accumulation of calcium phosphate may be a contributing factor to the death of heart tissue when cells are deprived of oxygen during a heart attack. Element mapping based on x-ray spectroscopy could verify and describe this type of pathological change.*

the ALS offer the greatest promise for high brightness. The ability to carry out spatially resolved spectroscopy for elements in this region would be of significant value.

A final area of promise at the ALS is biological x-ray crystallography. Most of the information that we have regarding the detailed structure of the two central classes of biological macromolecules—nucleic acids and proteins—is derived from analyses of the diffraction patterns produced by passing x-ray beams through crystalline arrays of these structures. In addition, our modern ideas about the transmission of genetic information, as well as our notions about the catalytic functions of enzymes, owe a great deal to x-ray crystallographic studies of biological macromolecules. An impressive example of x-ray diffraction work is the structure of the *ras* oncogene protein, described on pages 77–78.

Despite being optimized for the production of soft x-rays, the ALS is nonetheless well-suited for x-ray diffraction studies at the shorter wavelengths required for the satisfactory resolution of macromolecules. Indeed, careful comparisons have been carried out between the ALS Life Sciences wiggler and two established crystallography sources: one at Cornell University, the other at Stanford University. At a given photon energy, all three of these sources produce essentially the same flux, or rate of photon production; however, the brightness of the ALS source is significantly better (theoretically, as much as 100 times better) than the other two. Recently commissioned sources at the other facilities are brighter than the older sources, though still not as bright as the ALS wiggler is expected to be. For some experiments, flux is the more important factor, but in others brightness can give the ALS a considerable advantage.

The use of undulator radiation for soft x-ray scattering studies also offers great promise. The ability to match the wavelength of radiation in the soft x-ray and far UV regions of the spectrum to the characteristic sizes of various biological objects provides a powerful new probe into the microstructure of living organisms on a size scale ranging from one to hundreds of nanometers. In addition, the use of radiation with controlled polarization provides a way to study the symmetry and response of important biological systems.

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# *Research Medicine and Radiation Biophysics Division Publications*


Abbas, B., Hayes, T.L., Wilson, D.J. and Carr, K.E. (1989) Internal structure of the intestinal villus: Morphological and morphometric observations at different levels of the mouse villus. *J. Anat.*, **162**:263-273.

Aichelin, J., Cugnon, J., Fraenkel, Z., Frankel, K., Gale, C., Gyulassy, M., Keane, D., Ko, C.M., Randrup, J., Rosenhauer, A., Stöcker, H., Welke, G. and Wu, J.Q. (1989) Comparison of nuclear transport models with 800A-MeV La + La data. *Phys. Rev. Lett.*, **62**:1461-1464.

Alonso, J.R., Bercovitz, J., Chu, W.T., Ludewigt, B., Nyman, M., Singh, R.P., Stradtner, R., Tefalski, R. and Walton, R. (1989) Relocation of the helium ion radiotherapy program from the 184" synchrocyclotron to the BEVALAC. *Proc. of the 1989 IEEE Particle Accelerator Conference, Accelerator and Technology*, Chicago, p669-671.

Austin, M.A., Breslow, J.L., Hennekens, C.H., Buring, J.E., Willet, W.C. and Krauss, R.M. (1988) Low density lipoprotein subclass patterns and risk of myocardial infarction. *J. Am. Med. Assoc.*, **260**:1917-1921.

Austin, M.A., Brunzell, J.D., Fitch, W.L. and Krauss, R.M. (1990) Inheritance of low density lipoprotein subclass patterns in familial combined hyperlipidemia. *Atherosclerosis*, **10**:520-530.

Austin, M.A., King, M-C., Vranizan, K.M. and Krauss, R.M. (1990) Atherogenic lipoprotein phenotype: A proposed genetic marker for coronary heart disease risk. *Circulation*, **82**:495-506.

Ayyangar, K., Daftari, I., Palta, J. and Suntharalingam, N. (1989) Optimization of parameters for fitting linear accelerator photon beams using a modified CBEAM model. *Med. Phys.*, **16**:896-901.

Bacher, R., Blattmann, H., Boehringer, T., Coray, A., Egger, E., Pedroni, E., Scheib, S. and Phillips, M. (1990) Technical aspects and first results of discrete dynamic spot scanning with protons *Proc. of the International Heavy Particle Therapy Workshop* (Blattmann, H., ed.) PSI, Villigen, Switzerland, PSI-Bericht Nr. 69; p9-12.

Baker, J.R., Huesman, R.H. and Budinger, T.F. (1990) Linear analysis of rotationally invariant, radially variant tomographic imaging systems. *Proc. Medical Imaging IV Conference.*, Newport Beach, CA.

Bastacky, J., Goodman, C. and Hayes, T.L. (1990) A specimen holder for low-temperature scanning electron microscopy. *J. Electron Microsc. Tech.*, **14**:83-84.

Bastid, N., Alard, J.P., Arnold, J., Augerat, J., Babinet, R., Biagi, F., Brochard, F., Crouau, M., Charmensat, P., Dupieux, P., Fodor, Z., Fraysse, L., Girard, J., Gorodetzky, P., Gosset, J., Laspalles, C., Lemaire, M.C., LeMerdy, A., L'Hôte, D., Lucas, B., Marroncle, J., Montarou, G., Parizet, M.J., Poitou, J., Qassoud, D., Racca, C., Rahmani, A., Schimmerling, W., Terrien, Y. and Valette, O. (1990) Exclusive measurements of light fragment production at forward angles in Ne-Pb and Ne-NaF collisions at E/A=400 MeV and 800 MeV. *Nucl. Phys.*, **A506**:637-654.

Bland, P.K. and Williams, P.T. (1988) Activity patterns of men attending for fitness assessment. *Br. J. Sports Med.*, **22**:101-106.

Budinger, T.F. (1989) Nuclear medicine emission imaging: Principles and methods. (Margulis, Burhenne, eds.) *Alimentary Tract Radiol.*, St. Louis, p57-72.

Budinger, T.F. (1989) Perspectives on imaging modalities. *Cardiovasc. Intervent. Radiol.*, **12**:223-225.

Budinger, T.F. (1990) Advances in emission tomography: quo vadis? *J. Nucl. Med.*, **31**:628-631.

Carr, K.E., Hayes, T.L., Abbas, B. and Ainsworth, E.J. (1990) Collared crypts in irradiated small intestine. *J. Submicrosc. Cytol. Pathol.*, **22**:265-271.

Castro, J.R. (1989) Heavy charged particles in cancer treatment. In: *Cancer Management in Man* (Goldson, A.L., ed.) Dordrecht, The Netherlands: Kluwer Academic Publs., p179-182.

Castro, J.R., Blakely, E.A. and Linstadt, D.E. (1989) Particle therapy in treatment of urologic tumors. In: *Advances in Urologic Oncology: Treatment Perspectives* (Williams, R.D. and Carroll, P., eds.) Macmillian Publishing Co., NY, p149-160.

Castro, J.R., Collier, J.M., Petti, P.L., Nowakowski, V., Chen, G.T., Lyman, J.T., Linstadt, D., Gauger, G., Gutin, P. and Decker, M. (1989) Charged particle radiotherapy for lesions encircling the brain stem or spinal cord. *Int. J. Radiat. Oncol. Biol. Phys.*, **17**:477-484.

Castro, J.R., Nowakowski, V.N., Linstadt, D.E., Collier, J.M., Petti, P.L., Lyman, J.T., Phillips, T.L., Char, D., Gauger, G., Woodruff, K., Chu, W., Ludewigt, B., Renner, T., Singh, R.P., Pitluck, S. and Whitcomb, T. (1989) Heavy charged particle radiotherapy at the Lawrence Berkeley Laboratory. *Proc. of the EULIMA*

*Workshop on the Potential Value of Light Ion Beam Therapy* (Chauvel, P. and Wambersie, A., eds.) Nice, France, p219-232.

Char, D.H., Castro, J.R., Kroll, S.M., Irvine, A.R., Quivey, J.M. and Stone, R.D. (1990) Five-year follow-up of helium ion therapy for uveal melanoma. *Arch. Ophthalmol.*, **108**:209-214.

Char, D.H., Castro, J.R., Quivey, J.M., Phillips, T.L., Irvine, A.R., Stone, R.D. and Kroll, S. (1989) Uveal melanoma radiation. $^{125}$I brachytherapy versus helium ion irradiation. *Ophthalmol.*, **96**:1708-1715.

Chu, W.T. (1988) Strategies of building a light-ion synchrotron facility. *Proc. of the 1st Seoul International Symp. on Sciences and Technology of Heavy Ion Particle Accelerators*, Seoul, Korea, p123-148.

Chu, W.T. (1989) Physics and biomedical research using accelerated heavy ions. *Proc. of the '89 Summer Symp.*, Korean Federation of Science and Technology Societies; Seoul, Korea, p102-106.

Chu, W.T., Alonso, J.R., Halliwell, J.B., Ludewigt, B.A., Nyman, M.A., Renner, T.R., Singh, R.P., Stover, G.D. and Stradtner, R. (1990) Dynamic beam delivery methods. *Proc. of the International Heavy Particle Therapy Workshop* (Blattmann, H., ed.) PSI, Villigen, Switzerland, PSI-Bericht Nr. 69; p13-18.

Chu, W.T., Renner, T.R. and Ludewigt, B.A. (1989) Dynamic beam delivery for three-dimensional conformal therapy. *Proc. of the EULIMA Workshop on the Potential Value of Light Ion Beam Therapy*, (Chauvel, P. and Wambersie, A., eds.) Nice, France:, p295-328.

Cross, C.E., Forte, T., Stocker, R., Louie, S., Yamamoto, Y., Ames, B.N. and Frei, B. (1990) Oxidative stress and abnormal cholesterol metabolism in patients with adult respiratory distress syndrome. *J. Lab. Clin. Med.*, **115(4)**:396-404.

Dalal, K.B., Ebbe, S., Mazoyer, E., Carpenter, D. and Yee, T. (1990) Biochemical and functional abnormalities in hypercholesterolemic rabbit platelets. *Lipids*, **25**:86-92.

DeCampli, W., Moore, F.D. and Phillips, M.H. Radiation.(1988) In: *Exploring the Living Universe: A Strategy for Space Life Sciences.* Report of NASA Life Sciences Strategic Planning Study Committee.

Derenzo, S.E. (1990) *INTERFACING: A Laboratory Approach Using the Microcomputer for Instrumentation, Data Analysis and Control.* Prentice-Hall, Englewood Cliffs, NJ.

Derenzo, S.E. (1990) *Solutions Manual for INTERFACING: A Laboratory Approach Using the Microcomputer for Instrumentation, Data Analysis and Control.* Prentice-Hall, Englewood Cliffs, NJ.

Derenzo, S.E., Moses, W.W., Jackson, H.G., Turko, B.T., Cahoon, J.L., Geyer, A.B. and Vuletich, T.(1989) Initial characterization of a position-sensitive photodiode/ BGO detector for PET. *IEEE Trans. Nucl. Sci.*, **NS-36**:1084-1089.

Derenzo, S.E., Moses, W.W., Perera, R., Litton, J. and Cahoon, J. (1990) Prospects for new inorganic scintillators. *IEEE Trans. Nucl. Sci.*, **NS-37**:203-208.

Dicello, J.F., Lyman, J.T., McDonald, J.C. and Verhey, L.J. (1990) A portable system for microdosimetric intercomparisons by task group #20 of the American Association of Physicists in Medicine (AAPM). *Nucl. Instrum. Methods Phys. Res.*, **B45**:724-729.

Dreon, D.M., Frey, H.B., Ellsworth, N., Williams, P.T., Terry, R.B. and Wood, P.D. (1988) Dietary fat: Carbohydrate ratio and obesity in middle-aged men. *Am. J. Clin. Nutr.*, **47**:995-1000.

Dreon, D.M., Vranizan, K.M., Krauss, R.M., Austin, M.A. and Wood, P.D. (1990) The effects of polyunsaturated fat vs monounsaturated fat on plasma lipoproteins. *J. Am. Med. Assoc.*, **263**:2462-2466.

Dupieux, P., Alard, J.P., Augerat, J., Babinet, R., Bastid, N., Brochard, F., Charmensat, P., Marco, N.D, Fanet, H., Fodor, Z., Fraysse, L., Girard, J., Gorodetzky, P., Gosset, J., Laspalles, C., Lemaire, M.C., L'Hôte, D., Lucas, B., Marroncle, J., Montarou, G., Parizet, M.J., Poitou, J., Qassoud, D., Racca, C., Rahmani, A., Schimmerling, W. and Valette, O. (1988) Proton-proton correlations at small relative momentum in neon-nucleus collisions at E/A=400 and 800 MeV. *Phys. Lett.*, **B200**:17-20.

Durbin, P.W., Jeung, N., Rodgers, S.J., Turowski, P.N., Weitl, F.L., White, D.L. and Raymond, K.N. (1989) Removal of $^{238}$plutonium(IV) from mice by polycatecholate-, hydroxamate or -hydroxypyridinonate ligands. *Radiat. Prot. Dosim.*, **26**:351-358.

Durbin, P.W. and Schmidt, C.T. (1989) Predicting the kinetics of chelating agents in man from animal data. *Health Phys.*, **57**:165-174.

Durbin, P.W., White, D.L., Jeung, N.L., Weitl, F.L., Uhlir, L.C., Jones, E.S., Bruenger, F.W. and Raymond, K.N. (1989) Chelation of $^{238}$Pu(IV) *in vivo* by 3,4,3-LICAM(C): Effects of ligand methylation and pH. *Health Phys.*, **56**:839-855.

Ebbe, S. (1989) Animal models with inherited hematopoietic abnormalities as tools to study thrombopoiesis. *Blood Cells*, **15**:254-257.

Ebbe, S. (1990) Megakaryocyte size and ploidy in thrombocytopenic or megakaryocytopenic mice. In: *The Biology of Hematopoiesis* (Dainiak, N., Cronkite, E.P., McCaffrey, R. and Shadduck, R.K., eds.) Wiley-Liss, p133-144.

Ebbe, S., Carpenter, D. and Yee, T. (1989) Megakaryocytopenia in W/Wv mice is accompanied by an increase in size within ploidy groups and acceleration of maturation. *Blood*, **74**:94-98.

Ebbe, S., Yee, T. and Phalen, E. (1989) 5-Fluorouracil-induced thrombocytosis in mice is independent of the spleen and can be partially reproduced by repeated doses of cytosine arabinoside. *Exp. Hematol.*, **17**:822-826.

Ellett, W.H., Fabrikant, J.I. and Cooper, R.D. (1988) The BEIR IV Committee estimates of lung cancer mortality associated with exposure to radon progeny. *The 4th International Symp. on the Natural Radiation Environment, December 1987*, Lisbon, Portugal.

Endo, M., Chen, G.T., Collier, J.M. and Zink, S.R. (1988) An application of film dosimetry for helium ion radiotherapy. *Radiat. Med.*, **6**:141-145.

Erickson, S.K., Lear, S.R., Barker, M.E. and Musliner, T.A. (1990) Regulation of and cholesterol metabolism in the ethionine-induced premalignant rat liver. *J. Lipid Res.*, **31**:933-945.

Fabrikant, J.I. (1990) Factors that modify risks of radiation-induced cancer. *Health Phys.*, **59(1)**:77-87.

Fabrikant, J.I. (1990) Radon and lung cancer: the BEIR IV Report. *Health Phys.*, **59(1)**:89-97.

Fabrikant, J.I., Frankel, K.A., Phillips, M.H., Levy, R.P. and Lyman, J.T. (1989) Heavy-charged particle Bragg peak radiosurgery for intracranial arteriovenous malformations. *Proc. of the EULIMA Workshop on the Potential Value of Light Ion Beam Therapy* (Chauvel, P. and Wambersie, A., eds) Commission of the European Communities, Nice, France, p233-245.

Fabrikant, J.I., Levy, R.P., Frankel, K.A., Phillips, M.H. and Lyman, J.T. (1989) Neurosurgical application of ion beams. *Nucl. Instrum. Methods Phys. Res.*, **B40/41**:1376-1384.

Fabrikant, J.I., Levy, R.P., Frankel, K.A., Phillips, M.H., Lyman, J.T., Chuang, F.Y.S., Steinberg, G.K. and Marks, M.P. (1989) Stereotactic helium-ion radiosurgery for the treatment of intracranial arteriovenous malformations. *Proc. of the International Workshop of Proton and Narrow Photon Beam Therapy* (Heikkinen, E. and Kiviniitty, K., eds.) Univ. of Oulu, Oulu, Finland, p33-37.

Fein, G., Van, D.C., Davenport, L., Turetsky, B., Brant-Zawadzki, M., Zatz, L., Dillon, W. and Valk, P. (1990) Preservation of normal cognitive functioning in elderly subjects with extensive white-matter lesions of long duration. *Arch. Gen. Psychiatry*, **47**:220-223.

Forte, T.M., Davis, P.A. and Genzel-Boroviczeny, O. (1990) Lipoprotein metabolism during early development of the human. In: *Handbook of Human Growth and Developmental Biology*, Vol. II, Part B (Meisami, E. and Timiras, P.S., eds.) CRC Press, Inc., Boca Raton, FL, p297-322.

Forte, T.M., McCall, M.R., Amacher, S., Nordhausen, R.W., Vigne, J.L. and Mallory, J.B. Physical and chemical characteristics of apolipoprotein AI-lipid complexes produced by Chinese hamster ovary cells transfected with the human apolipoprotein AI gene. *Biochim. Biophys. Acta*, in press.

Forte, T.M., Genzel, B.O., Austin, M.A., Kao, L.C., Scott, C., Albers, J.J. and D'Harlingue, A.E. (1989) Effect of total parenteral nutrition with intravenous fat on lipids and high density lipoprotein heterogeneity in neonates. *J. Parenteral Enteral Nutr.*, **13(5)**:490-500.

Forte, T.M., McCall, M.R., Knowles, B.B. and Shore, V.G. (1989) Isolation and characterization of lipoproteins produced by human hepatoma-derived cell lines other than HepG2. *J. Lipid Res.*, **30(6)**:817-829.

Franceschini, G., Calabresi, L., Baio, M., Nichols, A.V. and Sirtori, C.R. (1989) Apolipoprotein AI-Milano: Mechanisms for the antiatherogenic potential. In: *Human Apolipoprotein Mutants: From Gene Structure to Phenotype Expression* (Sirtori, C.R., Francheschini, G., Brewer, Jr. H.B. and Assmann, G., eds.) Plenum Press, New York, p45-50.

Friedland, R.P., Jagust, W.J., Huesman, R.H., Koss, E., Knittel, B., Mathis, C.A., Ober, B.A., Mazoyer, B.M. and Budinger, T.F. (1989) Regional cerebral glucose transport and utilization in Alzheimer's disease. *Neurology*, **39**:1427-1434.

Gong, E.L., Nichols, A.V., Weisgraber, K.H., Forte, T.M., Shore, V.G. and Blanche, P.J. (1989) Discoidal complexes containing apolipoprotein E and their transformation by lecithin-cholesterol acyltransferase. *Biochim. Biophys. Acta*, **1006(3)**:317-328.

Gosset, J., Valette, O., Alard, J.P., Augera, J., Babinet, R., Bastid, N., Brochard, F., Marco, N.D., Dupieux, P., Fodor, Z., Fraysse, L., Gorodetzky, P., Lemaire, M.C., L'Hôte, D., Lucas, B., Marroncle, J., Montarou, G., Parizet, M.J., Poitou, J., Racca, C., Rahmani, A., Schimmerling, W. and Terrien, Y. (1989) Nuclear collective flow and charged pion emission in Ne-nucleus collisions at E/A = 800 MeV. *Phys. Rev. Lett.*, **62**:1251-1254.

Gregg, S.G., Mazzeo, R.S., Budinger, T.F. and Brooks, G.A. (1989) Acute anemia increases lactate production and decreases clearance during exercise. *J. Appl. Physiol.*, **67**:756-764.

Grierson, J.R., Link, J.M., Mathis, C.A., Rasey, J.S. and Krohn, K.A. (1989) A Radiosynthesis of fluorine-18 fluoromisonidazole. *J. Nucl. Med.*, **30**:343-350.

Grierson, J.R., Link, J.M., Mathis, C.A., Rasey, J.S. and Krohn, K.A. (1989) Radiosynthesis of [F-18] fluoromisonidazole. *J. Label. Compds. Radiopharm.*, **26**:6-7.

Griffin, T.W., Wambersie, A., Laramore, G. and Castro, J. (1988) International clinical trials in radiation oncology. High LET: Heavy particle trials. *Int. J. Radiat. Oncol. Biol. Phys.*, **14**(Supp 1):S83-92.

Haber, S., Derenzo, S.E. and Uber, D. (1990) Positron range deblurring in high resolution positron emission tomography. *IEEE Tran. Nucl. Sci..*, **NS-37**:1301-1307.

Hill, R.J., Leven, R.M., Levin, F.C. and Levin, J. (1989) The effect of partially purified thrombopoietin on guinea pig megakaryocyte ploidy *in vitro*. *Exp. Hematol.*, **17**:903-907.

Innerarity, T., Mahley, R.W., Weisgraber, K.H., Bersot, T.P., Krauss, R.M., Vega, G.L., Grundy, S.M., Friedl, W., Davignon, J. and McCarthy, B.J. (1990) Familial defective apolipoprotein B-100: a mutation of apolipoprotein B that causes hypercholesterolemia. *J. Lipid Res.*, **31**:1337-1350.

Ishida, B.Y., Albee, D. and Paigen, B. (1990) Interconversion of prebeta-migrating lipoproteins containing apolipoprotein A-I and HDL. *J. Lipid Res.*, **31**:227-236.

Ishida, B.Y., Frolich, J. and Fielding, C.J. (1987) Prebeta-migrating high density lipoprotein: Quantitation in normal and hyperlipidemic plasma by solid phase radioimmunoassay following electrophoretic transfer. *J. Lipid Res.*, **28**:778-786.

Ishida, B.Y. and Paigen, B. (1989) Atherosclerosis in the mouse. *Genetic Factors in Atherosclerosis: Approaches and Model Systems* (Sparks, R.S. and Lusis, A.J., eds.) Karger, NY, p189-222.

Jagust, W.J., Davies, P., Tiller, B.J. and Reed, B.R. (1990) Focal Alzheimer's disease. *Neurology*, **40**:14-29.

Jagust, W.J., Reed, B.R., Seab, J.P., Kramer, J.H. and Budinger, T.F. (1989) Clinical-physiologic correlates of Alzheimer's disease and frontal lobe dementia. *Am. J. Physiol. Imaging*, **4**:89-96.

Johnson, M.P., Mathis, C.A., Shulgin, A.T., Hoffman, A.J. and Nichols, D.E. (1990) [$^{125}$I]-2-(2,5-dimethoxy-4-iodophenyl)aminoethane ([$^{125}$I]-2C-I) as a label for the 5-$HT_2$ receptor in rat frontal cortex. *Pharmacol. Biochem. Behav.*, **35**:211-217.

Keltner, J.T., Roos, M.S., Brakeman, P.R. and Budinger, T.F. Magnetohydrodynamics of blood flow. *Mag. Res. Med.*, in press.

Kindy-Degnan, N., Char, D.H., Castro, J.R., Kroll, S., Stone, R.D., Quivey, J.M., Phillips, T.L. and Irvine, A.R. (1989) Effect of various doses of radiation for uveal melanoma on regression, visual acuity, complications and survival. *Am. J. Ophthalmol.*, **107**:114-120.

Kirz, J., Ade, H., Anderson, E., Attwood, D., Buckley, C., Hellman, S., Howells, N., Jacobsen, C., Dern, D., Lindaas, S., McNulty, I., Oversluizen, M., Rarback, H., Rivers, M., Rothman, S., Sayre, D. and Shu, D. X-ray microscope with the NSLS soft x-ray undulator. *Physica Scripta*, in press.

Krauss, R.M. Hyperlipidemia. In: *Current Therapy in Endocrinology and Metabolism*, 4th edition, B.C. Decker, Inc., in press.

Krauss, R.M. The tangled web of coronary risk factors. *Am. J. Med.*, in press.

Krauss, R.M. The metabolic impact of oral contraceptives. *Am. J. Obstet. Gynecol.*, in press.

Krauss, R.M. (1988) The effects of oral contraceptives on plasma lipids and lipoproteins. *Int. J. Fertil.*, **33** (Suppl):35-42.

Krauss, R.M., Grunfeld, C., Doerrler, W.T. and Feingold, F.R. (1990) Tumor necrosis factor acutely increases plasma levels of very low density lipoproteins of normal size and composition. *Endocrinology*, **127**:1016-1021.

Krauss, R.M. (1989) Clinical significance of low density lipoprotein subclasses. *Perspect. Lipid Disorders*, **6**:4-8.

Krauss, R.M. (1989) Exercise, lipoproteins and coronary artery disease. *Circulation*, **79**:1143-1145.

Krauss, R.M. (1989) Lipoproteins and coronary heart disease. *Postgrad. Med.*, Spec No. 54-58; discussion 89-90.

La Belle, M. and Krauss, R.M. (1990) Differences in carbohydrate content of low density lipoproteins associated with low density lipoprotein subclass patterns. *J. Lipid Res.*, **31**:1577-1588.

La Belle, M., McCall, M.R., Krauss, R.M. and Forte, T.M. (1990) Unique structural properties of LDL produced by several human hepatoma-derived cell lines. *Biochim. Biophys. Acta*, **1046**:288-293.

La Belle, M., Mok, K.L., Karu, A.E. and Krauss, R.M. Increased immunoreactivity of apoprotein B epitopes during prolonged storage of low density lipoproteins. *Clin. Chim. Acta*, in press.

Ladias, J.A., Kwiterovich, P.J., Smith, H.H., Miller, M., Bachorik, P.S., Forte, T., Lusis, A.J. and Antonarakis, S.E. (1989) Apolipoprotein B-100 Hopkins (arginine4019-tryptophan). A new apolipoprotein B-100 variant in a family with premature atherosclerosis and hyperapobetalipoproteinemia. *J. Am. Med. Assoc.*, **262**:1980-1988.

Leven, R. (1989) Elutriation for isolation of megakaryocytes. *Blood Cells*, **15**:306-313.

Leven, R.M. and Yee, T. (1990) Collagenase production by guinea pig megakaryocytes *in vitro*. *Exp. Hematol.*, **18**:743-747.

Levy, R.P., Fabrikant, J.I., Frankel, K.A., Phillips, M.H. and Lyman, J.T. (1989) Stereotactic heavy-charged-particle Bragg peak radiosurgery for the treatment of intracranial arteriovenous malformations in childhood and adolescence. *Neurosurgery*, **24**:841-852.

Levy, R.P., Fabrikant, J.I., Lyman, J.T., Frankel, K.A., Phillips, M.H., Lawrence, J.H. and Tobias, C.A. (1989) Clinical results of stereotactic helium-ion radiosurgery of the pituitary gland at Lawrence Berkeley Laboratory. *Proc. of the International Workshop of Proton and Narrow Photon Beam Therapy* (Heikkinen, E. and Kiviniitty, K., eds.) Univ. of Oulu, Oulu, Finland, p38-42.

Liburdy, R.P., Burgess, N.L., Bartley, J.C. and Mindich, B.P. (1988) Quantitative HPLC analysis of human plaque proteins in coronary and thoracic aorta arteries. *Atherosclerosis*, **71**:103-112.

Lo, E.H., Frankel, K.A., Delapaz, R.L., Poljak, A., Woodruff, K.H., Brennan, K.M., Phillips, M.H., Valk, P.E., Steinberg, G.K. and Fabrikant, J.I. (1989) Cerebrovascular and metabolic perturbations in delayed heavy charged particle radiation injury. *Brain Res.*, **504**:168-172.

Lovato, A.A., Char, D.H., Castro, J.R. and Kroll, S.M. (1989) The effect of silicone nasolacrimal intubation on epiphora after helium ion irradiation of uveal melanomas. *Am. J. Ophthalmol.*, **108**:431-434.

Lyman, J., Phillips, M., Frankel, K., Fabrikant, J. and Levy, R. Heavy charged-particle beams for stereotactic radiosurgery. (1989) *Proc. of the International Workshop of Proton and Narrow Photon Beam Therapy* (Heikkinen, E. and Kiviniitty, K., eds.) Univ. of Oulu, Oulu, Finland, p43-47.

Lyman, J.T. Evaluation and optimization of treatment plans using a biological probabilistic objective function. (1990) *Proc. of the International Heavy Particle Therapy Workshop* (Blattmann, H., ed.) PSI, Villigen, Switzerland, PSI-Bericht Nr. 69; p61-64.

Lyman, J.T., Phillips, M.H., Frankel, K.A. and Fabrikant, J.I. (1989) Stereotactic frame for neuroradiology and charged-particle Bragg peak radiosurgery of intracranial disorders. *Int. J. Radiat. Oncol. Biol. Phys.*, **16**:1615-1621.

Lyman, J.T. and Wolbarst, A.B. (1989) Optimization of radiation therapy, IV: A dose-volume histogram reduction algorithm. *Int. J. Radiat. Oncol. Biol. Phys.*, **17**:433-436.

Mathis, C.A., Gerdes, J.M., Shulgin, A.T., Hoffman, A.J. and Nichols, D.E. (1989) Synthesis of high specific activity radiohalogenated amphetamine analogues for studies of the serotonin 5-$HT_2$ receptor. *J. Label. Compds. Radiopharm.*, **26**:130-132.

McCall, M.R., Nichols, A.V., Blanche, P.J., Shore, V.G. and Forte, T.M. (1989) Lecithin: Cholesterol acyltransferase-induced transformation of HepG2 lipoproteins. *J. Lipid Res.*, **30(10)**:1579-1589.

McKenna, D.J., Nazarali, A.J., Hoffman, A.J., Nichols, D.E., Mathis, C.A. and Saavedra, J.M. (1989) Common

receptors for hallucinogens in rat brain: A comparative autoradiographic study using [$^{125}$I]LSD and [$^{125}$I]DOI, A new psychotomimetic radioligand. *Brain Res.*, **476**:45-56.

Moses, W.W. (1990) Performance of a coincidence based blood activity monitor. *IEEE Trans. Nucl. Sci.*, **NS-37**:580-584.

Moses, W.W. and Derenzo, S.E. (1989) Cerium fluoride, A new heavy, fast scintillator. *IEEE Trans. Nucl. Sci.*, **NS-36**:173-176.

Moses, W.W. and Derenzo, S.E. (1990) Lead Carbonate, A new fast, heavy scintillator. *IEEE Trans. Nucl. Sci.*, **NS-37**:96-100.

Moses, W.W. and Derenzo, S.E. (1990) A method for measuring the time structure of synchrotron x-ray beams. *Nucl. Instrum. Methods Phys. Res.*, **A291**:435-437.

Moses, W.W., Derenzo, S.E., Geyer, A.B., Huesman, R.H. and Uber, D.C. (1989) Tuning algorithms used by the Donner 600 crystal tomograph. *IEEE Trans. Nucl. Sci.*, **NS-36**:1025-1029.

Newmark, R.D., Un, S., Williams, P.G., Carson, P.J., Morimoto, H. and Klein, M.P. (1990) $^{3}$H Nuclear magnetic resonance study of anaerobic glycolysis in packed erythrocytes. *Proc. Natl. Acad. Sci., USA*, **87**:583-587.

Nichols, A.V. Conversions in the origins and metabolism of human plasma HDL. In: *Advances in Cholesterol Research* (Swaney, J.B. and Esfahani, M. eds.) Telford Press, Caldwell, NJ, in press.

Nichols, A.V. and Gong, E.L. Origins and determinants of HDL populations and their subpopulations. *Proc. of the Karolinska Hospital Symposium on Disorders of HDL* (Carlson, L., ed.), in press.

Nichols, A.V., Gong, E.L., Blanche, P.J. and Forte, T.M. (1989) HDL populations: Origins and interconversions. *Proc. of the 2nd International Workshop on HDL and Atherosclerosis* (Miller, N.E., ed.) Elsevier, Amsterdam, p159-171.

Nichols, A.V., Cheung, M.C., Blanche, P.J., Gong, E.L., Francheschini, G. and Sirtori, C.R. (1989) Apolipoprotein-specific high density lipoprotein populations in plasma of carriers of the apolipoprotein AI-Milano. In: *Human Apolipoprotein Mutants: From Gene Structure to Phenotype Expression* (Sirtori, C.R., Francheschini, G., Brewer, Jr., H.B. and Assmann, G., eds.) Plenum Press, New York, p67-73.

Nichols, A.V., Blanche, P.J., Shore, V.G. and Gong, E.L. (1989) Conversion of apolipoprotein-specific high-density lipoprotein populations during incubation of human plasma. *Biochim. Biophys. Acta*, **1001**:325-337.

Paigen, B., Ishida, B.Y., Verstuyft, J., Winters, R.B. and Albee, D. (1990) Atherosclerosis susceptibility differences among progenitors of recombinant inbred strains of mice. *Arteriosclerosis*, **10**:316-323.

Palta, J.R., Ayyangar, K.M., Daftari, I.K. and Suntharalingam, N. (1990) Characteristics of photon beams from Philips SL25 linear accelerators. *Med. Phys.*, **17**:106-116.

Palta, J.R., Daftari, I.K. and Suntharalingam, N. (1988) Field size dependence of wedge factors. *Med. Phys.*, **15**:624-626.

Palta, J.R., Daftari, I.K., Ayyangar, K.M. and Suntharalingam, N. (1990) Electron beam characteristics on a Philips SL25. *Med. Phys.*, **17**:27-34.

Parizet, M.J., Alard, J.P., Rahmani, A., Montarou, G., Augerat, J., Bastid, N., Demaison, P., Dupieux, P., Fraysse, L., Marroncle, J., Raha, S., Babinet, R., Fodor, Z., Gosset, J., L'Hôte, D., Poitou, J., Schimmerling, W., Terrien, Y., Valette, O., Lemaire, M.C., Gorodetzky, P. and Racca, C. (1989) Proton production in relativistic heavy ion collisions; comparison with a thermodynamical model. *Int. J. Modern Phys. A.* **4**:3689-3703.

Patz, S., Wong, S.T. and Roos, M.S. (1989) Missing pulse steady-state free precession. *Magn. Reson. Med.*, **10**:194-209.

Pedroni, E., Bacher, R., Blattmann, H., Boehringer, T., Corey, A., Egger, E., Phillips, M. and Scheib, S. (1990) The 200 MeV proton therapy project at PSI: A status report. *Proc. of the International Heavy Particle Therapy Workshop* (Blattmann, H., ed.) PSI, Villigen, Switzerland, PSI-Bericht Nr **69**:1-8.

Phillips, M.H., Frankel, K.A., Lyman, J.T., Fabrikant, J.I. and Levy, R.P. (1989) Comparison of different radiation types and irradiation geometries in stereotactic radiosurgery. *Proc. of the EULIMA Workshop on the Potential Value of Light Ion Beam Therapy* (Chauvel, P. and Wambersie, A., eds.) Nice, France, p271-294.

Phillips, M.H., Frankel, K.A., Lyman, J.T., Fabrikant, J.I. and Levy, R.P. (1989) Heavy-charged-particle stereotactic radiosurgery: Cerebral angiography and CT in the treatment of intracranial vascular malformations. *Int. J. Radiat. Oncol. Biol. Phys.* **17**:419-426.

Phillips, M.H., Frankel, K.A., Lyman, J.T., Fabrikant, J.I. and Levy, R.P. (1990) Comparison of different radiation types and irradiation geometries in stereotactic radiosurgery. *Int. J. Radiat. Oncol. Biol. Phys.*, **18**:211-220.

Phillips, M.H., Frankel, K.A., Tjoa, T., Lyman, J.T., Fabrikant, J.I. and Levy, R.P. (1990) Effects of target size on the comparison of photon and charged particle dose distributions. *Proc. of the International Heavy Particle Therapy Workshop* (Blattmann, H., ed.) PSI, Villigen, Switzerland, PSI-Bericht Nr., **69**:24-26.

Reed, B.R., Jagust, W.J. and Seab, J.P. (1989) Mental status as a predictor of daily function in progressive dementia. *Gerontologist*, **29**:804-807.

Reed, B.R., Jagust, W.J., Seab, J.P. and Ober, B.A. (1989) Memory and regional cerebral blood flow in mildly symptomatic Alzheimer's disease. *Neurology*, **39**:1537-1539.

Renner, T.R., Chu, W.T., Ludewigt, B.A., Halliwell, J., Nyman, M.A., Singh, R.P., Stover, G.D. and Stradtner, R. (1989) Preliminary results of a raster scanning beam delivery system. *Proc. of the 1989 IEEE Particle Accelerator Conference, Accelerator and Technology.* Chicago, p672-674.

Renner, T.R., Chu, W.T., Ludewigt, B.A., Nyman, M.A. and Stradtner, R. (1989) Multisegmented ionization chamber dosimetry system for light ion beams. *Nucl. Instrum. Methods Phys. Res.*, **A281**:640-648.

Roos, M.S., Apfel, R.E. and Wardlaw, S.C. (1988) Application of 30-MHz acoustic scattering to the study of human red blood cells. *J. Acoust. Soc. Am.*, **83**:1639-1644.

Roos, M.S. and Wong, S.T.S. (1990) Stochastic NMR imaging: Spatial localization with oscillating gradients. *J. Mag. Res.*, **87**:554-566.

Ross, S.M. (1990) Combined DC and ELF magnetic fields can alter cell proliferation. *Bioelectromagnetics*, **11**:27-36.

Ross, S.M., Ferrier, J.M. and Aubin, J.E. (1989) Studies on the alignment of fibroblasts in uniform applied electrical fields. *Bioelectromagnetics*, **10**:371-384.

Ross, S.M., Joshi, R. and Frank, C.B. (1990) Establishment and comparison of fibroblast cell lines from the medial collateral and anterior cruciate ligaments of the rabbit. *In Vitro Cell Dev. Biol.*, **26**:579-584.

Rothman, S.S., Iskander, N., Attwood, D., Vladimirsky, Y., McQuaid, K., Grendell, J., Kirz, J., Ade, H., McNulty, I. and Kern, D. (1989) The interior of a whole and unmodified biological object—The zymogen granule—viewed with a high-resolution x-ray microscope. *Biochim. Biophys. Acta*, **991**:484-486.

Rothman, S.S. and Melese, T. (1988) "Leaky" cells of glandular epithelia. *Int. Rev. Cytol.*, **112**:225-244.

Rubin, E.M., Ishida, B., Clift, S. and Krauss, R.M. Expression of human apolipoprotein A-I transgenic mice results in reduced plasma levels of murine apolipoprotein A-I and the appearance of two new HDL size subclasses. *Proc. Natl. Acad. Sci., USA*, in press.

Rubinsky, B., Lee, C.Y., Bastacky, J. and Onik, G. (1990) The process of freezing and the mechanism of damage during hepatic cryosurgery. *Cryobiology*, **27**:85-97.

Salen, G., Shore, V.G., Tint, G.S., Forte, T.M., Shefer, S., Horak, I., Horak, E., Dayal, B., Nguyen, L., Batta, A.K., et al. (1989) Increased sitosterol absorption, decreased removal and expanded body pools compensate for reduced cholesterol synthesis in sitosterolemia with xanthomatosis. *J. Lipid Res.*, **30(9)**:1319-1330.

Sargent, T., III, Braun, U., Braun, G., Kusubov, N. and Bristol, K.S. (1989) Cerebral and peripheral demethylation of psychotomimetics measured by expired $^{14}CO_2$. *Nucl. Med. Biol.*, **16**:91-99.

Schimmerling, W. and Curtis, S.B. (1989) Workshop summary: Biomedical and space-related research with heavy ions at the BEVALAC. *Radiat. Res.*, **119**:193-204.

Schimmerling, W., Miller, J., Wong, M., Rapkin, M., Howard, J., Spieler, H.G. and Jarrett, B.V. (1989) The fragmentation of 670A-MeV neon-20 as a function of depth in water; I: experiment. *Radiat. Res.*, **120**:36-71.

Steinberg, G.K., Fabrikant, J.I., Marks, M.P., Levy, R.P., Frankel, K.A., Phillips, M.H., Shuer, L.M. and Silverberg, G.D. (1990) Stereotactic heavy-charged-particle Bragg peak radiosurgery for intracranial vascular malformations. *N. Engl. J. Med.*, **323**:96-101.

Superko, H.R., Wood, P.D. and Krauss, R.M. (1989) Effect of alpha- and selective beta-blockade for hypertension control on plasma lipoproteins, apoproteins, lipoprotein subclasses and postprandial lipemia. *Am. J. Med.*, **86**:26-31.

Terry, R.B., Wood, P.D., Haskell, W.L., Stefanick, M.L. and Krauss, R.M. (1989) Regional adiposity patterns in relation to lipids, lipoprotein cholesterol and lipoprotein subfraction mass in men. *J. Clin. Endocrinol. Metab.*, **68**:191-199.

Thomas, R.G., Durbin, P.W., McInroy, J.F. and Healy, J.W. (1989) Estimation of human gonadal Pu and Ce concentrations from animal data. *Health Phys.*, **57**:97-107.

Tobias, C.A., Stansbury, H.E., Chu, W.T. and Gough, R.A. (1989) LIBRA: A Progress Report. *Proc. of the EULIMA Workshop on the Potential Value of Light Ion Beam Therapy* (Chauvel, P. and Wambersie, A., eds.) Nice, France, p373-380.

Upton, A.C., Becker, B.B., Clifton, K.H., Denniston, C., Epp, E.R., Fabrikant, J.I., Grahn, D., Hall, E.J., Harte, D.L., Herbert, D.E., Hoel, D.G., Howe, G.H., Jablon, S., Kennedy, A.R., Knudson, A.G., Jr. and Thomas, D.C. (1990) *The Health Effects of Exposure to Low Levels of Ionizing Radiation, BEIR V*. Washington D.C., National Academy Press.

Vega, G.L., Krauss, R.M. and Grundy, S.M. (1990) Pravastatin therapy in primary moderate hypercholesterolemia: Changes in metabolism of apolipoprotein B-containing lipoproteins. *J. Intern. Med.*, **227**:81-94.

Verdery, R.B., Benham, D.F., Baldwin, H.L., Goldberg, A.P. and Nichols, A.V. (1989) Measurement of normative HDL subfraction cholesterol levels by Gaussian summation analysis of gradient gels. *J. Lipid Res.*, **30**:1085-1095.

Weisgraber, K.H., Innerarity, T.L., Newhouse. Y.M., Young, S.G., Arnold, K.S., Krauss, R.M., Vega, G.L., Grundy, S.M. and Mahley, R.W. (1988) Familial defective apolipoprotein B-100: Enhanced binding of monoclonal antibody MB47 to abnormal low density lipoproteins. *Proc. Natl. Acad. Sci., USA*, **85**:9758-9762.

Williams, P.T. (1990) Weight set-point theory and the high-density lipoprotein concentrations of long-distance runners. *Metabolism*, **39**:460-467.

Williams, P.T. (1990) Weight set-point theory predicts HDL-cholesterol levels in previously obese long-distance runners. *Int. J. Obes.*, **14**:421-427.

Williams, P.T., Alber, J.J., Krauss, R.M. and Wood, P.D.S. (1990) Associations of lecithin: Cholesterol acyltransferase (LCAT) mass concentrations with exercise, weight loss, and plasma lipoprotein subfraction concentration in men. *Atherosclerosis*, **82**:53-58.

Williams, P.T., Krauss, R.M., Nichols, A.V., Vranzanan, K.M. and Wood, P.D. (1990) Identifying the predominant peak diameter of high-density (HDL) and low-density (LDL) lipoproteins by electrophoresis. *J. Lipid Res.*, **31**:1131-1139.

Williams, P.T., Krauss, R.M., Vranizan, K.M., Albers, J.J., Terry, R.B. and Wood, P.D. (1989) Effects of exercise-induced weight loss on low density lipoprotein subfractions in healthy men. *Arteriosclerosis*, **9**:623-632.

Williams, P.T., Krauss, R.M., Vranizan, K.M. and Wood, P.D. (1990) Changes in lipoprotein subfractions during diet-induced and exercise-induced weight loss in moderately overweight men. *Circulation*, **81**:1293-1304.

Wong, S.T.S., Roos, M.S., Newmark, R.D. and Budinger, T.F. (1990) Discrete analysis of stochastic NMR I. *J. Mag. Res.*, **87**:242-264.

Wong, S.T.S., Roos, M.S., Newmark, R.D. and Budinger, T.F. (1990) Discrete analysis of stochastic NMR II. *J. Mag. Res.*, **87**:265-286.

Wood, P.D., Stefanick, M.L., Dreon, D.M., Frey, H.B., Garay, S.C., Williams, P.T., Superko, H.R., Fortmann, S.P., Albers, J.J. and Vranizan, K.M. (1988) Changes in plasma lipids and lipoproteins in overweight men during weight loss through dieting as compared with exercise. *N. Engl. J. Med.*, **319**:1173-1179.

Yager, D., Butler, J.P., Bastacky, J., Israel, E., Smith, G. and Drazen, J.M. (1989) Amplification of airway constriction due to liquid filling of airway interstices. *J. Appl. Physiol.*, **66**:2873-2884.

Young, S.G., Witztum, J.L., Carew, T.E., Krauss, R.W. and Lindgren, F.T. (1989) Colestipol-induced changes in LDL composition and metabolism. II. Studies in humans. *J. Lipid Res.*, **30**:225-238.

# CHEMICAL BIODYNAMICS

THE LABORATORY OF CHEMICAL BIODYNAMICS was established in 1945 to conduct basic research on the dynamics of living cells and on the interaction of radiant energy with organic matter. Now a Division of LBL, as well as an Organized Research Unit of the Berkeley campus, LCB is making important advances in three areas: photosynthesis, structural biology, and energy storage. The use of biophysical techniques to study the molecular mechanisms of photosynthesis continues to be a central theme of the Division, as it has been throughout its history. Our new efforts in this area are turning to the use of molecular genetic and structural determination methods to probe questions of molecular structure and function.

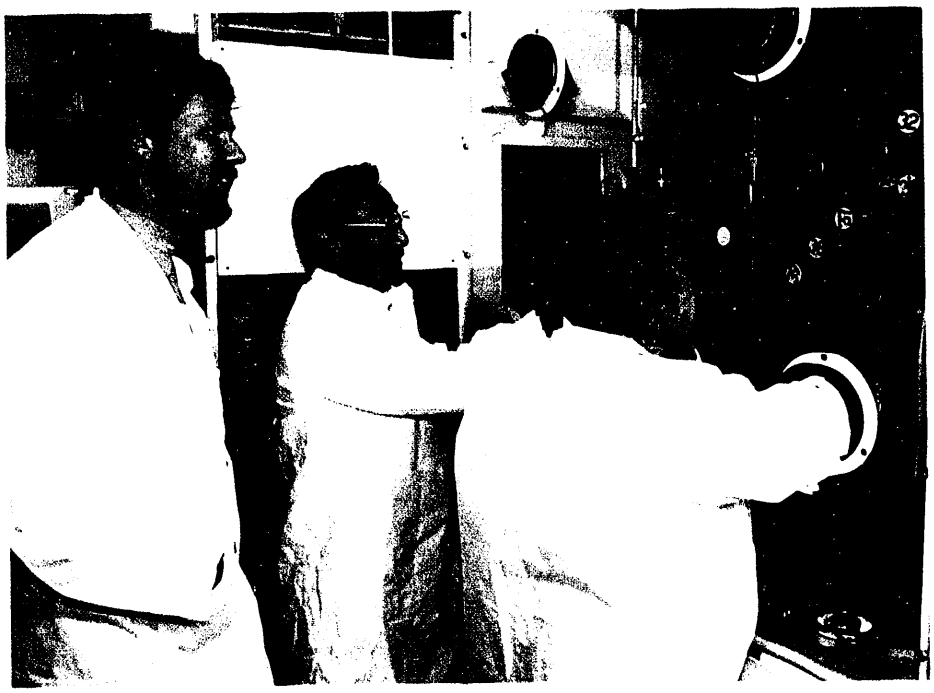
*The Chemical Biodynamics Division is making important advances in three areas: photosynthesis, structural biology, and energy storage.*

In a second area, both nuclear magnetic resonance (NMR) and x-ray crystallographic techniques are being complemented by high-resolution electron paramagnetic resonance (EPR) and x-ray absorption spectroscopy to probe the structure of complex biomolecules. And the diverse tools of modern molecular biology are being used to explore the broad questions implied by the term *structural biology*. We are interested, for example, in how the functional activity of genes is regulated by both the primary and higher-order structure of normal and damaged DNA, as well as by interactions with proteins and other nucleic acids. In our work on catalytic antibodies, we have been able to tailor the structure of proteins so that the molecules carry out selected chemical reactions.

A third area of effort encompasses the study of the fundamental chemistry of electronically excited molecules, an area that underlies any understanding of the energy storage processes in photosynthesis. We are working not only toward the use of sophisticated chemistry to store the energy of photons, but also toward the development of systems that actually mimic the photosynthetic apparatus in the trapping and transfer of energy.

The Chemical Biodynamics Division is also the home of the National Tritium Labeling Facility (NTLF). The NTLF was established in 1982, and it continues to serve as one of the few facilities in the nation equipped to label compounds to very high specific activities of tritium ($^3H$). It thus serves as a laboratory where researchers from across the U.S. can carry out labeling and radiopurification procedures that would be impossible at their home institutions. The mandated functions of the NTLF are to engage in research and development of advanced labeling techniques and to disseminate the results, to promote collaborative research using labeled molecules, to provide labeling services to the nation's scientists, and to train researchers in labeling methodologies. One of the NTLF's most important activities is to supply labeled biomolecules for tritium-NMR spectroscopy, a key technique in our structural biology research.

*ChemBio researcher Jim Bartholomew and Albany, California, high school science teacher Carroll Coughlin look over an autoradiograph showing bands of DNA. Coughlin will incorporate ideas and discoveries from the frontiers of science into her courses.*



*Phil Williams (left) and Hiromi Morimoto of LBL's National Tritium Labeling Facility oversee the work of Ann Cross of Yale University, who is carrying out a tritium-labeling operation inside a glove box. At the NTLF, visiting researchers can prepare and purify their own tritiated compounds.*

## PHOTOSYNTHESIS AND BIOLOGICAL ENERGY

*The Chemical Biodynamics Division traces its roots to early studies of carbon metabolism in photosynthesis, and significant effort continues to be directed toward an understanding of the dynamics of this process, perhaps the most fundamental in the biosphere . Ongoing studies focus on the light reactions of photosynthesis and on the organization of photosynthetically active membranes. Complementary investigations take a molecular genetic approach to understanding structural and functional aspects of photosynthesis; for example, we are seeking to sequence the genes responsible for synthesis of the photosynthetic apparatus and to elucidate the means by which these genes are regulated. We are also exploring the activities of the enzymes responsible for photosynthetic pigment biosynthesis. Still other studies aim at a broader understanding of plant biochemistry.*

### The Reactions of Photosynthesis

The conversion of sunlight to chemical energy in green plants is our principal source of biological energy. In this process, carbon dioxide is "reduced" to form sugar, and water is "oxidized" to form molecular oxygen. Photosynthesis begins with photon capture by chlorophyll molecules. These photons cause electrons in chlorophyll to be raised to higher energy levels. The excited electrons are then passed along a chain of acceptor molecules; ultimately, their energy is used to drive the oxidation/reduction reactions of photosynthesis.

To further our understanding of this critical natural process, we are using spectroscopic and other biophysical approaches to investigate the mechanisms of photon capture, excitation transfer, and energy trapping by chlorophyll and other photosynthetic pigments. Our interests include the earliest steps of electron transfer and the mechanism by which water is oxidized to molecular oxygen. During the past year, we continued to study fast reactions by means of optical and EPR spectroscopy, and we used EPR and x-ray absorption spectroscopy to study the structural features of key molecules in photosynthesis. We also looked at the higher-order structure and composition of photosynthetic complexes and thylakoid membranes (sites of photon capture in chloroplasts), using biochemical and molecular genetic techniques. Finally, we investigated several model compounds capable of the light-induced electron transfer that lies at the heart of photosynthetic energy conversion, and we looked at compounds that are candidates for storing electrons in the water-oxidation reaction.

As an example of this interrelated program of research, several significant findings have emerged from our studies of photosystem II, the green-plant enzyme that converts water to oxygen during photosynthesis. It is known that photosystem II contains a molecular complex of four manganese (Mn) atoms that are involved in electron transfer when oxygen is produced from water. To study this complex and the process of oxygen evolution, we have pioneered the use of x-ray edge spectroscopy and a technique called extended x-ray absorption fine structure (EXAFS), in conjunction with low-temperature EPR measurements. EXAFS studies, in fact, were the first to indicate the presence of a bridged manganese structure in the oxygen-evolving complex. We have now obtained EXAFS spectra for spinach photosystem II particles stabilized in various oxidation states; these spectra illuminate the structure of the manganese complex and the changes that occur during the photosystem II enzymatic cycle. To our surprise, the Mn atoms are not the only sites of electron storage in photosystem II. This led us to postulate that a ligand to Mn, perhaps histidine (an amino acid) in another protein associated with photosystem II, might also be an electron storage site. Electron spin echo spectroscopy of photosystem II membranes derived from the thermophilic cyanobacterium *Synechococcus* grown on nitrogen-15 has provided us with the first direct evidence to support such a hypothesis. Further, comparison of photosystem II complexes from spinach and *Synechococcus*, using EXAFS and EPR, show striking similarities. These results suggest that the structure of the manganese complex has been largely conserved throughout the full evolutionary history of oxygen-evolving photosynthetic species.

### Molecular Genetics of Photosynthesis

We are conducting molecular genetic studies on the photosynthetic bacterium *Rhodobacter capsulatus*. The genome of *R. capsulatus* contains a single region that codes for most of the proteins known to be essential to photosynthesis. This includes reaction center proteins, a long-wavelength light-harvesting antenna, and the enzymes required for synthesis of bacteriochlorophyll and carotenoid pigments. Our aims in studying the genes in this cluster are to determine the complete sequence of nucleotide building

blocks, to characterize the activities of the pigment-synthesis enzymes, and to understand how the genes are regulated. *R. capsulatus* is an ideal organism for such studies, because it has the unusual ability to switch from respiratory metabolism to photosynthetic metabolism when oxygen becomes scarce. This characteristic facilitates identification and propagation of photosynthetic mutants.

*ChemBio scientist Marie Alberti prepares to load a sample of DNA into a gel electrophoresis system used for sequencing. Alberti, John Hearst, and Greg Armstrong have identified and mapped the genes that control the production of carotenoids, the yellowish-red pigments that play a key role in the lives of plants and animals.*

One highlight of these studies has been the sequencing of eight *R. capsulatus genes* that control the production of carotenoids—yellowish to red pigments that provide protection against the destructive effects of oxygen and sunlight. Without carotenoids, the powerful photochemical reactions involved in the photosynthetic process would destroy a plant rather than sustain it. In addition, carotenoids increase the efficiency of photosynthesis by absorbing wavelengths of light that are not readily absorbed by chlorophyll. Despite the crucial roles of these pigments, no carotenoid gene from any organism had previously been sequenced, nor had the molecular mechanisms regulating carotenoid production been elucidated. After obtaining the sequence, we were able to identify a previously unknown gene. We also identified possible promoters. These are short strings of DNA that mark the spot where transcription (synthesis of RNA from a DNA template) should begin. At least two of the eight genes were preceded by promoters similar to those found in the bacterium *E. coli*. Finally, we found a possible binding site for proteins that regulate the amount of RNA produced by certain carotenoid genes.

**Plant Biochemistry**

We are exploring both structural and functional aspects of the plant protein-pigment phytochrome, which controls all developmental aspects of plant growth. Inactive phytochrome undergoes a photochemical interconversion to a new, active form; however, the structural features of this interconversion, as well as other aspects of phytochrome structure, remain unknown. Among our aims, then, are to determine the conformation of the pigment-protein linkage in phytochrome and to determine the structural changes in both pigment and protein that accompany the change from inactive to active form. Understanding the underlying mechanisms by which phytochrome functions may allow us to control plant growth, flowering, and fruiting.

## STRUCTURAL BIOLOGY

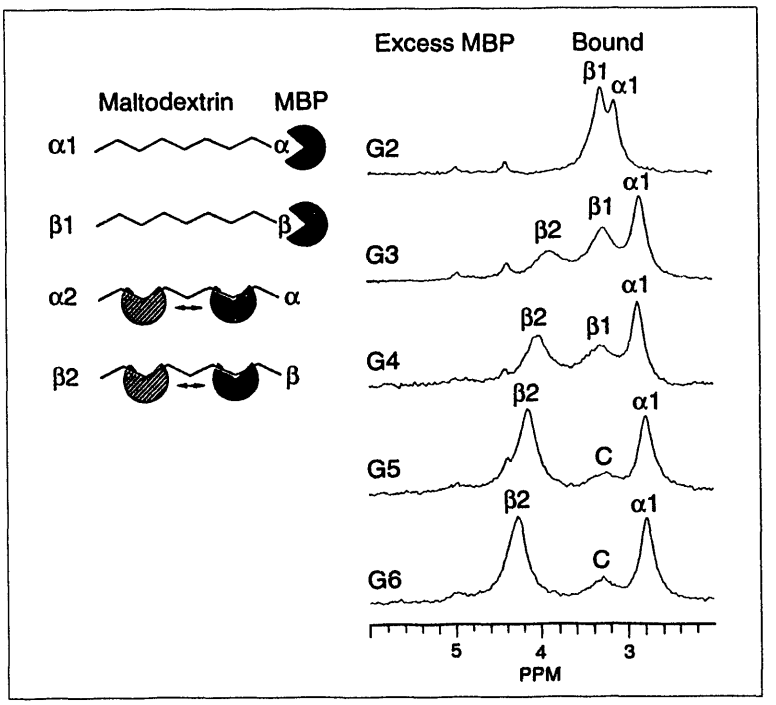
*Modern structural biology has come to encompass far more than "classical" high-resolution studies of biomolecular structure. The farther-reaching interest today is to relate structure to biological function to understand how primary and higher-order molecular structure, as well as larger-scale organizational structure, affect fundamental cellular processes such as enzyme catalysis, mutagenesis, and DNA synthesis. Active research programs in the Chemical Biodynamics Division well reflect this broadening scope of structural biology. The tools of these programs include NMR and x-ray crystallography, as well as photochemical and molecular genetic techniques. The subjects of study include both nucleic acids and proteins. In addition, aptly symbolic of our aim to understand how biomolecules work in terms of their structure, we are pursuing a goal of designing and synthesizing catalytic antibodies that carry out specific, predetermined reactions.*

### NMR Studies

To examine the relationships between structure and function, we have continued our applications of NMR to complex biological molecules. In this technique, nuclei with an unpaired proton or neutron absorb radio waves of characteristic frequency in a magnetic field. Such nuclei have nonzero angular momentum, or spin. Our NMR projects include analysis of proteins, as well as small DNA and RNA molecules. It has been possible to use just proton NMR for some studies, but for others we have taken advantage of better resolution with nuclei with other spins.

A special capability at LBL is tritium NMR, due to the presence of the National Tritium Labeling Facility. We have taken advantage of this to examine how a bacterial protein called maltose-binding protein, or MBP, binds to maltodextrins—the carbohydrates that it helps transport across the cell membrane. Maltodextrins are chains of the simple sugar glucose; we studied MBP binding to maltodextrins that contained two to six glucose subunits and were labeled at one site each with tritium. The terminal glucose subunit can have either of two structures, desginated alpha and beta. When a maltodextrin has three or more subunits, MBP can attach either to a central glucose or to the terminal glucose. As it turns out, MBP preferentially binds an alpha terminal glucose; this is the tightest binding mode, and with time all maltodextrins become trapped in this form. In the case of beta maltodextrins, MBP preferentially binds a central glucose, and when there are four or more units in the chain, this binding is a dynamic process in which the central glucose units slide through the binding site. In addition to having intrinsic interest, this system serves as a prototype for studying the details of how proteins and nucleic acids bind smaller molecules for a variety of purposes.

The first two levels of nucleic-acid structure are primary structure (sequence of nucleotide building blocks) and secondary structure (regions where "base pairing" occurs between the nucleotides). We have been using



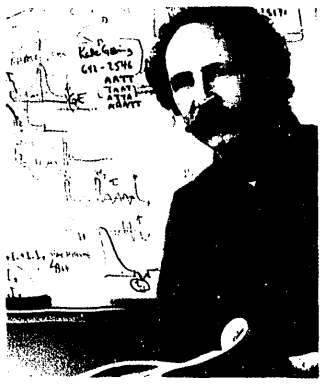

*Binding of maltose-binding protein (MBP) to maltodextrins, carbohydrates that consist of chains of the simple sugar glucose. (Left) MBP preferentially binds an alpha (α) terminal glucose. When the terminal glucose has the beta (β) structure, MBP preferentially binds a central glucose. (Right) Tritium spectra showing binding of MBP to maltodextrins containing two to six glucose units (G2 to G6). Binding to a central glucose is a dynamic process in which the glucose units slide through the binding site.*

NMR to study an autocatalytic, or self-cleaving, RNA that belongs to a class of catalytic molecules called hammerheads because their proposed secondary structure resembles a hammer. The autocatalytic reaction breaks down a long chain of duplicate RNA sequences into messenger RNA molecules that are used in protein synthesis. Through a series of small changes in sequence, we made an RNA with a very well-resolved imino proton (N-H) NMR spectrum. From this we have shown that the proposed hammerhead structure is basically correct, although some of the base pairs are not stable in this form under normal conditions. We have also compared a normal RNA, which had undergone the self-cleavage reaction, with two slowly cleaving forms. The uncleaved molecules showed similar secondary structure. Both the normal and slowly cleaving forms gave similar NMR spectral changes when titrated with magnesium ions, which are required for the cleavage reaction. In a related study we showed that the introduction of a single deoxyribose sugar at the cleavage site (instead of the normal ribose in RNA) completely stopped the cleavage reaction. Studies are underway to further define the structural requirements for the self-cleavage reaction.

Like proteins, RNA must fold correctly to interact with other molecules and to perform its many biological functions. Hairpin loops are important elements of the three-dimensional structure of large RNAs. We have determined the structure of a very stable and very common RNA hairpin by NMR spectroscopy. The sequence of nucleotides in the hairpin occurs very often in a variety of RNAs and may serve both as an initiation site for RNA folding and as a protein recognition site. By revealing interactions of hydrogen atoms with each other and with phosphorus atoms, NMR spectroscopy allowed a high-resolution structure for the hairpin to be derived. Extensive base stacking and other structural features of the loop explain the unusual hairpin stability and suggest why reverse transcriptase (the enzyme that synthesizes DNA from an RNA template) cannot read through the loop, although it can transcribe other kinds of secondary RNA structure.

Another application of NMR spectroscopy is in studies of cellular metabolism in living organisms. Phosphorus-31 NMR, for example, has

evolved into an important means for determining concentrations of phosphorylated metabolites and is now moving into the clinical arena. Among our early contributions to this field, we demonstrated the feasibility of implanting radio-frequency coils around the organs of laboratory animals to permit the recording of NMR spectra over long periods of time. Using this technique, we subsequently described phosphorus exchange reactions in the rat heart and kidney and demonstrated that there are pools of metabolic intermediates not directly visible in conventional high-resolution NMR spectra.

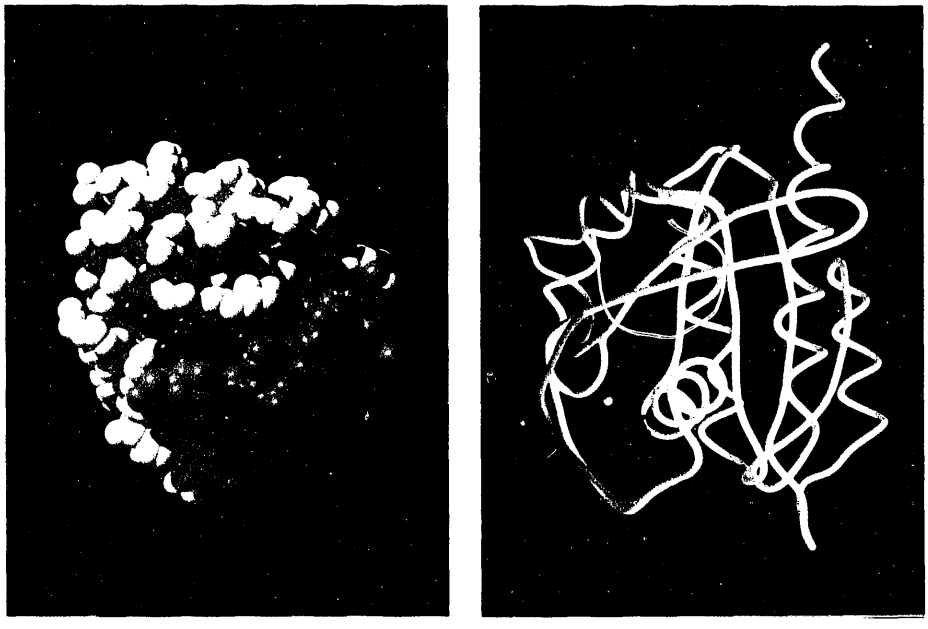


*ChemBio researcher David Wemmer has demonstrated the binding states of maltose-binding protein (MBP), a bacterial protein that helps transport carbohydrates across the cell membrane.*

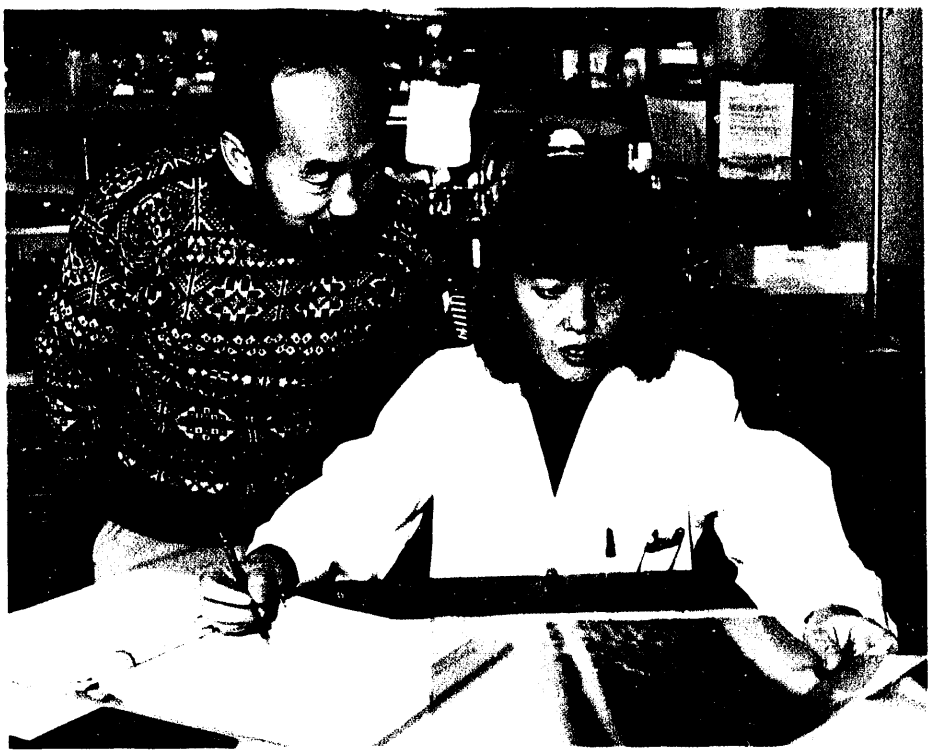
### X-Ray Crystallographic Studies

A key crystallographic result recently provided an elegant illustration of the aim of modern structural biology—to elucidate the intricacies of biological function by probing biological structure. By determining the crystal structure of a protein responsible for cancerous cell proliferation in a class of human tumors, a reasonable explanation could be proposed for the functional difference between the oncogenic (cancer-causing) and normal forms.

The *ras* oncogene is one of the most commonly found oncogenes in human cancer cells. A single point mutation (which results in production of a protein with a single amino-acid substitution) is sufficient to convert the normal protein encoded by the *ras* gene to an oncogenic form. In one such mutant, the amino acid valine replaces glycine at position 12. The abnormal protein, when complexed with guanosine triphosphate (GTP), is thought to sustain a signal cascade inside the cell that leads to cell proliferation. The normal protein, on the other hand, converts GTP to GDP (guanosine diphosphate) after only a brief time and thus turns the proliferation signal off.



*Computer-generated space-filling model (left) and backbone structure (right) of the human* ras *protein. This protein acts as a molecular switch signalling cell growth and differentiation. Damage to the structure results in the unregulated cell growth associated with cancer. The switching depends on the state of a guanine nucleotide (darkest area in both figures).*



*With Rosalind Kim, ChemBio Division Director Sung-Hou Kim reviews the sequence of DNA from a genetically engineered gene. Genetic-engineering techniques are used in a wide range of studies in the Division.*

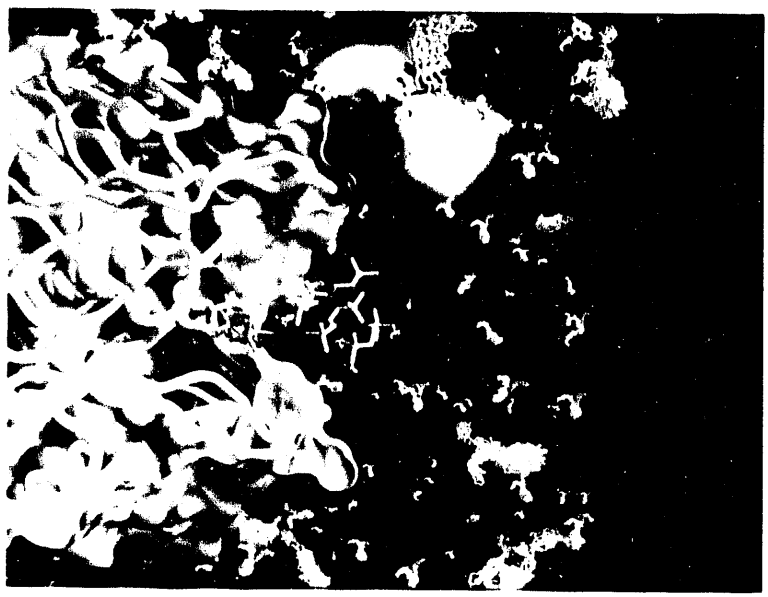
We have refined both the normal and the oncogenic ras protein structures to a resolution of 2.2 Å. These high-resolution pictures, which reveal atomic details of three-dimensional structure, illustrate the structural differences between the normal and cancerous forms of the protein. These studies will provide the foundation for understanding how the *ras* protein functions and aid in the design of drugs that may suppress *ras*-mediated human tumors.

### Synthesis of Catalytic Antibodies

As well as having important biomedical applications, the ability to design and synthesize enzymes that carry out specific, predetermined reactions would represent a dramatic step toward the commercialization of many chemical processes. Key to the design of such selective catalysts is the generation of highly specific binding sites. It is now possible to generate such binding sites, with enzymelike affinity and specificity, as parts of monoclonal antibodies produced by living cells. A monoclonal antibody recognizes and binds to just one type of molecule, or "antigen." For example, monoclonal antibodies have been produced against nucleic acids, proteins, carbohydrates, steroids, and synthetic polymers.

Because an antibody binding site can be generated to virtually any molecule of interest, the development of general strategies for introducing cata-

lytic activity into antibodies could provide tailor-made catalysts for use in biology, chemistry and medicine. Mutation of specific, selected gene sites is being explored as a strategy for introducing catalytic groups into antibody binding sites. During the past year, we have generated antibodies that contain chemical groups capable of catalyzing isomerization (rearrangement of atoms within a molecule), carbon-carbon bond formation, and hydrolytic cleavage (splitting by addition of water) of cabon-nitrogen bonds. We are also exploring strategies for generating antibodies to catalyze selective cleavage of the bonds that link the amino acids in proteins. Finally, a new strategy is being developed for antigen-antibody recognition. The strategy involves attaching small molecules with desired antigenic properties to proteins.



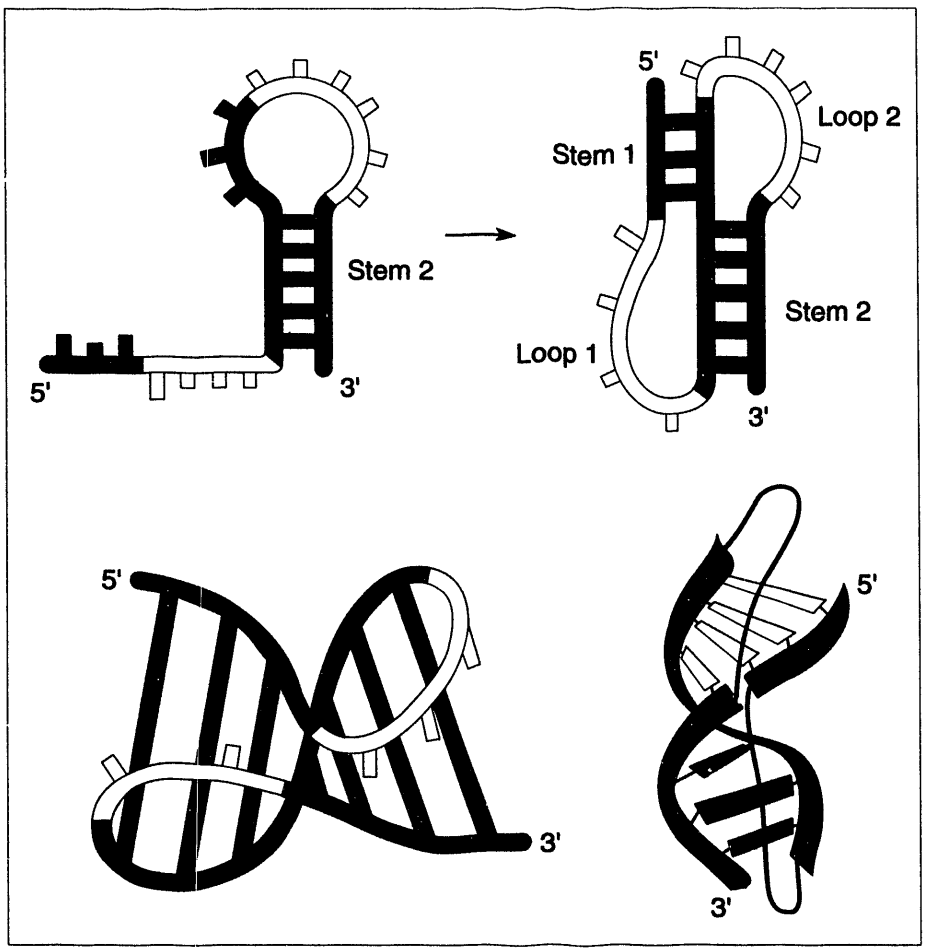
*Computer image of an antibody that catalyzes the cleavage of chemical bonds in choline esters and carbohydrates by addition of water. This is the first catalytic antibody characterized by Peter Schultz's group in ChemBio. Photo by Art Olson, Scripps College.*

**Understanding Structure and Function: Other Approaches**

A number of other investigations are also under way, with the common goal of understanding the structure and function of biological macromolecules. These studies include research on the mechanism of repair of DNA c[illegible]age, the structure of catalytically active RNA, the mechanisms by which DNA synthesis is controlled in human cells, the structure of telomeres (the ends of chromosomes), and the tertiary structure of RNA "pseudoknots." In addition to catalytic RNAs, our studies of RNA structure cover ribosomal RNA (rRNA) and transfer RNA (tRNA)—molecules that play crucial roles in protein synthesis. We have also developed a differential polarization microscope that reveals chiral structures (which exhibit right- or left-handedness) and ordered linear structures in single living cells.

The studies of catalytic RNA illustrate particularly well the close relationship between an understanding of structure and insights into biological function. When first transcribed from DNA, many messenger RNA (mRNA) molecules contain intervening nucleotide sequences called introns, which must be removed before the message is translated into protein. Whereas it was once thought that all catalytic activity in the cell depended on proteins, it is now known that intron removal can be a self-catalyzed reaction—that is, the RNA itself can serve as a catalyst. Furthermore, it is now thought that the three-dimensional structure of the RNA is the key to unerring recognition of the cutting sites.

*A schematic illustration of an RNA pseudoknot structure. The two stems of the pseudoknot stack atop one another to form an apparent single stem; the pseudoknot thus mimics tRNA.*




We are taking several approaches to determining the secondary structure, as well as tertiary structure (three-dimensional folding), of catalytic RNAs. One method is to photochemically crosslink the molecule with psoralen. The RNA can then be digested and the crosslinked fragments identified. The technique has already revealed several three-dimensional structural features of M1 RNA, the essential catalytic component of the enzyme RNase P (which occurs in living cells as an RNA/protein complex), and work continues toward refining the structure further. A more theoretical approach to structure determination is being taken to uncover the folding pattern of 16S ribosomal RNA from *E. coli*. We are using available data, including proposed secondary structural features, in connection with an energy-modeling computer program to determine the most energetically favorable—that is, the most stable—conformations. In a preliminary study using this approach, we determined the tertiary structure of a yeast tRNA. Subsequent x-ray crystallography results confirmed our conclusion.

The many biological functions of RNA, including its enzymatic activities and its regulatory roles in protein synthesis, are made possible by its tertiary structure. However, whereas secondary structure can be predicted by

consideration of free-energy (energy made available by a chemical or structural change), little is known about the tertiary structure of RNA—with the exception of tRNA. We thus undertook a study of pseudoknots—a proposed tertiary structure on the ends of many plant viral RNAs. Pseudoknotting allows the virus to fold itself so that it looks like tRNA. During the past year, using NMR and other techniques, we characterized the pseudoknot conformation. The deduced structure shows that the two stems of the pseudoknot stack atop one another to form what appears to be one continuous, longer stem. The stem has a standard double-helix structure, explaining why the pseudoknot can fool enzymes that recognize tRNA, which also has a double-helix structure.

Another study involves the dynamics of DNA in concentrated solutions. This is of special biological importance because many cellular systems have a very high concentration of nucleic acid. Fluorescent labeling was used to study the diffusion of both isolated and clumped strands of entangled phage (bacterial virus) DNA in concentrated solutions. It was found that as concentration increases, the diffusion of isolated strands decreases, whereas the diffusion of clumped strands increases. These data are in qualitative agreement with a body of theoretical work and with results obtained in recent light-scattering studies of concentrated DNA solutions.

## CHEMISTRY OF ENERGY STORAGE

*Photosynthesis is a natural process of great biochemical interest—and potentially of great practical interest as well. To mimic photosynthesis would allow us to trap the energy of the sun in chemical form, where it could be stored indefinitely. This remains a distant goal, but we are ever mindful of it in our efforts to gain a fundamental understanding of electronically excited molecules, and especially the ways in which photon energy is stored in the form of high-energy chemical bonds. Our programs include attempts to find suitable catalysts and photosensitizers (substances that cause increased sensitivity to light) of the component reactions of photosynthesis, as well as basic optical and infrared spectroscopic studies on the use and storage of the sun's most abundant type of energy—red and near infrared light .*

### Artificial Photosynthesis

The ultimate solution to the problem of dwindling fossil fuel supplies would be the ability to convert solar energy directly into chemical fuels. The natural model is photosynthesis. Photosynthetic organisms are able to store the energy of captured photons as chemical potential by exploiting both a phase boundary that allows for charge separation and a set of chemical compounds that can use the separated charge to oxidize water and to reduce an "acceptor" to fuel. An artificial photosynthetic system would thus require both a suitable phase boundary and a proper combination of

catalysts and photosensitizers. We have focused on the second half of this problem, in particular studying systems that could be used either to catalyze the oxidation of water or the reduction of carbon dioxide.

The oxidation of water to molecular oxygen in photosynthesis involves a polynuclear manganese complex. We have therefore synthesized a variety of manganese-amine large-ring complexes, including two of special interest. Both undergo reversible one-electron oxidations and quasi-reversible one-electron reductions. In addition, both complexes interact with water when they are oxidized and may serve as model systems for the multiple-electron oxidation of water.

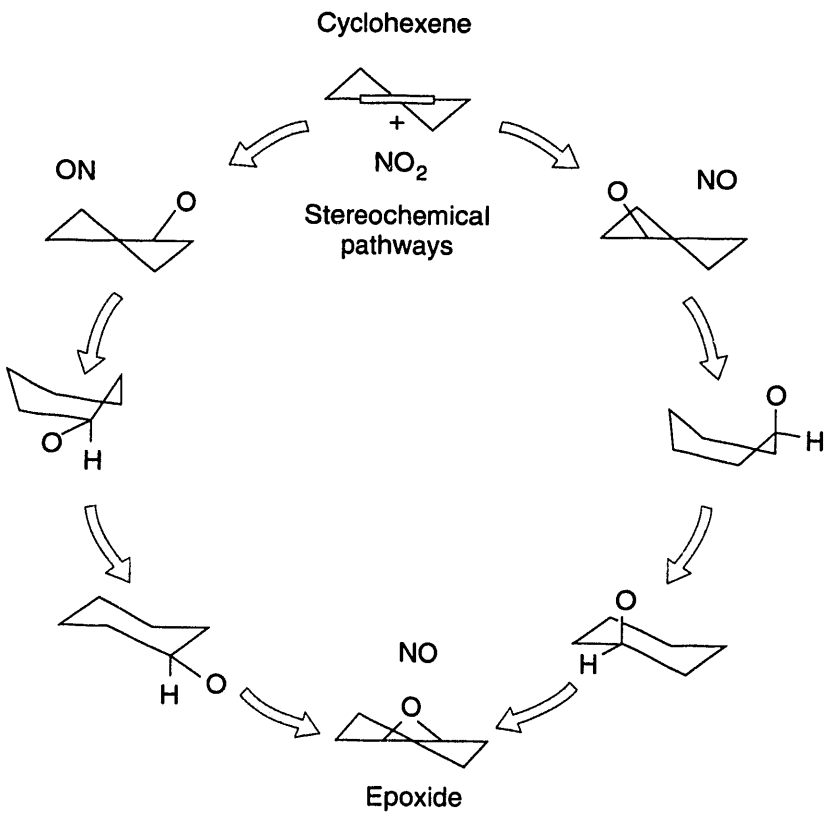
We have also been studying amine-metal large-ring complexes as catalysts for the reduction of carbon dioxide. For example, we have used such complexes as catalysts in aqueous photochemical systems that also contain photosensitizers and an electron donor. Irradiation with blue light reduces carbon dioxide to carbon monoxide and produces hydrogen gas as a by-product. The efficiency of photoreduction is a function of solution acidity and temperature. It is obvious that the reduction is a complicated, multiple-event reaction, and we are continuing our efforts to understand the relationship between the conformations of these complexes and their catalytic selectivity and reactivity.

**Basic Photochemistry**

Chemical reactions that can be initiated by red and near infrared light are crucial for substantial improvement of the use and storage of solar energy, because 75 percent of solar radiation lies in this range. Identifying chemistry that can be induced with these long wavelengths is one of the main goals of our work. We focus our efforts on chemical reactions that suggest new concepts for light-assisted synthesis of high-valued chemicals from abundant ones, for photocatalysis, and for temporary chemical storage of near infrared photons and conversion of their energy into electricity. Chemistry initiated by long-wavelength light allows tight product control and opens up opportunities for new product-specific chemical syntheses whose significance goes beyond solar photochemistry.

Currently, oxidized hydrocarbons are made expensively or nonspecifically from petroleum. As a first step toward developing a new, commercially useful technique for producing these compounds, we used red laser light to demonstrate photooxidation of alkenes and alkynes (hydrocarbons with double or triple bonds, respectively). Sufficiently long wavelengths of red light resulted in the formation of specific chemical products. The experiments were done at 12 degrees Kelvin in a matrix of solid argon or xenon. For photooxidation by red light to be commercially viable, the technique must be developed for use at higher temperatures. We are now trying to do the same experiments in a matrix of solid carbon dioxide. Ultimately, we plan to use a polymer matrix at room temperature.

Each hydrocarbon in the matrix was paired with a nitrogen dioxide molecule. In the photooxidation reactions, an oxygen atom was transferred from the nitrogen dioxide to the hydrocarbon. Nitrogen dioxide can be regenerated in a susbsequent oxidation reaction involving molecular oxy-




*In a reaction induced by red light, an oxygen atom is transferred from nitrogen dioxide ($NO_2$) to either of two positions on a cyclohexene molecule. A series of intermediate products forms via two distinct stereochemical pathways. The end result of both pathways is a single epoxide product.*

gen; thus, oxygen can serve as the "terminal oxidant." The wavelengths of red light effective in generating a single product were much longer than the threshold wavelength for dissociation of nitrogen dioxide to release free oxygen atoms.

Infrared spectroscopy and laser-induced photolysis (initiation of a reaction by light) were used to demonstrate the step-by-step stereochemical, or three-dimensional, pathways of the reactions. Photooxidation of alkenes produced epoxides, substances in which an oxygen atom forms a bridge between two carbon atoms. Epoxides serve as building blocks for plastics and other polymers. In the case of cyclohexene, we observed two distinct intermediate products, although just one epoxide ultimately formed. Oxidation of alkynes produced ketenes (molecules in which an oxygen atom is double-bonded to a carbon atom, which in turn is double-bonded to another carbon). When propyne, which has three carbon atoms, was oxidized, the first step was to add an oxygen atom to either the central or end carbon to form a "ketocarbene." This is the first time ever that researchers haved observed the structure of the first product of an alkyne oxidation. Although two different ketocarbenes formed as intermediates, just one final ketene product was generated.

We have demonstrated the feasibility of chemically storing the energy of near infrared light and converting it into electrical energy. Infrared photons were used to excite molecular oxygen to its singlet state. The singlet

oxygen was then inserted into an acceptor molecule for storage; the energy was retrieved by conversion to electricity at a silicon electrode. We are also studying infrared-initiated oxidation/reduction reactions in aqueous and colloidal semiconductor solutions. Crucial for progress here is the elucidation of the reaction steps and precise knowledge of the energetics and rate of each of them. During the past year, we have investigated the steps of photooxidation of iodine and bromine ions at titanium oxide and zinc oxide colloidal particles. Using near infrared absorption spectroscopy, we have established that the detailed mechanism involves formation of a surface-adsorbed iodine or bromine atom that subsequently reacts with a compound of one of these elements from solution. The efficiency of the latter step appears to be limited by the ability of the surface-adsorbed atom to recapture an electron. This work illustrates another approach to temporary storage of infrared photons and efficient conversion into useful energy.

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## *Chemical Biodynamics Division Publications*


Andres, H., Morimoto, H. and Williams, P.G. (1990) Preparation and use of $LiEt_3BT$ and $LiAlT_4$ at maximum specific activity. *J. Chem. Soc. Chem. Comm.*, p627-628.

Armstrong, G.A., Alberti, M., Leach, F. and Hearst, J.E. (1989) Nucleotide sequence, organization, and nature of the protein products of the carotenoid biosynthesis gene cluster of *Rhodobacter capsulatus*. *Mol. and Gen. Genet.*, **216**:254-268.

Brewer, K.J., Calvin, M., Otvos, J.W. and Spreer, L.O. (1989) Synthesis, structure, and characterization of a mixed-valence Mn(III), Mn(IV) di-m-oxo complex with a macrocyclic tetraaza ligand. *Inorg. Chem.*, **28**:4446.

Britt, R.D., DeRose, V.J., Yachandra, V.K., Kim, D.H., Sauer, K. and Klein, M.P. (1990) Pulsed EPR studies of the manganese center of the oxygen-evolving complex of photosystem II. In: *Current Research in Photosynthesis* (Baltsheffsky, M., ed.) Kluwer Academic Publs., Dordrecht, The Netherlands, **Vol I**:769-772.

Britt, R.D. and Klein, M.P. (1988) A versatile loop-gap resonator probe for low temperature electron spin echo studies. *J. Mag. Res.*, **74**:535-549.

Britt, R.D., Sauer, K. and Klein, M.P. (1989) Pulsed EPR studies of the oxygen-evolving manganese complex of photosystem II. In: *Current Research in Photosynthesis.* (Baltscheffsky, M., ed.) Kluwer, Amsterdam. **Vol I(3)**:760-772.

Britt, R.D., Zimmermann, J.L., Sauer, K. and Klein, M.P. (1989) ESEEM studies of ammonia binding to the catalytic Mn of the water oxidation complex of photosystem II. In: *Pulsed EPR: A New Field of Applications* (Keijzers, C.P., Reijerse, E.J. and Schmidt, J., eds.) North Holland, Amsterdam, p150-155.

Britt, D., Zimmermann, J.L., Sauer, K. and Klein, M.P. (1989) Ammonia binds to the catalytic Mn of the oxygen-evolving complex of photosystem II: Evidence by electron spin-echo envelope modulation spectroscopy. *J. Am. Chem. Soc.*, **111**:3522-3532.

Calvin, M. (1989) Forty years of photosynthesis and related activities. *Photosyn. Res.*, **21**:1.

Calvin, M. (1989) Intermediates in the photosynthetic carbon cycle: An essay. *Biochim. Biophys. Acta.*, **1000**.

Calvin, M. and Taylor, S.E. (1989) Fuels from algae. In: *Algal Biotechnology.* (Cresswell, R.C., Rees, T.A.V. and Shah, N., eds.) Longmans, Green, London, England.

Cheng, S., Gamper, H.B. and Hearst, J.E. (1989) RecA-directed hybridization of psoralen-monoadducted DNA

oligonucleotides to duplex targets. In: *Biochemical Probes in Biochemistry*. (Nielsen, P.E., ed.) Kluwer Academic Publs., Boston, MA.

Cheong, C., Tinoco, Jr., I. and Chollet., A. (1988) Thermodynamics of base pairing involving 2,6-diaminopurine. *Nucl. Acids Res.*, **16**:5115-5122.

Cook, D.N., Armstrong, G.A. and Hearst, J.E. (1989) Induction of anaerobic gene expression in *Rhodobacter capsulatus* is not accompanied by a local change in chromosomal supercoiling as measured by a novel assay. *J. Bacter.*, **171**:4836-4843.

Dawson, M.I., Hobbs, P.D., Dorsky, A. and Morimoto, H. (1990) The synthesis of [1, 3 -$^3$H]-4-(4'-Azido-5',6',7',8'-tetrahydro-5',5',8'8'-tetramethyl-2'-anthracenyl) benzoic acid as a probe of the retinoic acid receptor. *J. Label. Compds. Radiopharm.*, **28(1)**:89-98.

Dexheimer, S.L., Gohdes, J.W., Chan, M.K., Hagen, K.S., Armstrong, W.H. and Klein, M.P. (1989) Detection of EPR spectra in S=2 states in trivalent manganese complexes. *J. Am. Chem. Soc.*, **111**:8923-8925.

Dexheimer, S.L. and Klein, M.P. (1988) Sensitivity improvement of a varian E-109 EPR spectrometer with a low noise microwave amplifier. *Rev. Sci. Instr.*, **59**:764-766.

Dexheimer, S.L., Sauer, K. and Klein, M.P. (1990) Parallel polarization EPR studies of the oxygen-evolving complex of photosytem II. In: *Current Research in Photosynthesis* (Baltsheffsky, M., ed.) Kluwer Academic Publs., Dordrecht, The Netherlands. **Vol. I(3)**:761-764.

Dorsky, A.M., Morimoto, H., Saljoughian, M., Williams, P.G. and Rapoport, H. (1989) An introduction to the National Tritium Labeling Facility. *Synthesis and Applications of Isotopically Labelled Compounds 1988 (Proc. Third Int. Symp., Innsbruck)*. (Baillie, T.A. and Jones, J.R., eds.) Elsevier, Amsterdam, p541-544.

Frei, H., Fitzmaurice, D.J. and Gratzel, M. (1990) Surface chelation of semiconductors and interfacial electron transfer. *Langmuir*, **6**:198-206.

Frei, H. and Pimentel, G.C. (1989) Chemistry on ground state and excited electronic surfaces induced by selective photo-excitation in matrices. In: *Chemistry and Physics of Matrix Isolated Species* (L. Andrews and M. Moskovits, eds.) Elsevier, Amsterdam, p139-165.

Garnett, J.L., Long, M.A., Chit, T. and Williams, P.G. (1990) Homogeneous and heterogeneous metal catalyzed hydrogen isotope exchange of nitro-benzene with tritium gas and tritiated water. *J. Chem. Soc., Faraday Trans.*, **86(5)**:875-879.

Gingrich, J.C., Gasparich, G.E., Sauer, K. and Bryant, D.A. (1990) Nucleotide sequence and expression of the two genes encoding the D2 protein and the single gene encoding the CP43 protein of photosystem II in the cyanobacterium *Synechococcus* sp. PCC 7002. *Photosyn. Res.*, **24**:137-150.

Gratzel, M. and Frei, H. (1989) Evidence for hole injection from $O_2(^1D)$ into p-Si. *J. Phys. Chem.*, **93**:7037-7041.

Guiles, R.D., DeRose, V.J., Yachandra, K., Zimmermann, J.L., Sauer, K. and Klein, M.P. (1989) Structures and oxidation states of manganese in several S-states of photosystem II determined by x-ray absorption spectroscopy. In: *Current Research in Photosynthesis*. (Baltscheffsky, M., ed.) Kluwer, Amsterdam, **Vol I(3)**:789-792.

Guiles, R.D., Yachandra, V.K., McDermott, A.E., DeRose, V.J. Zimmermann, J.L., Sauer, K. and Klein, M.P. (1990) Structures and oxidation states of Mn in several S-states of photosystem II determined by x-ray absorption spectroscopy. In: *Current Research in Photosynthesis* (Baltsheffsky, M., ed.) Kluwer Academic Publs., Dordrecht, The Netherlands, **Vol I**:789-792.

Guiles, R.D., Yachandra, V.K., McDermott, A.E., Cole, J.L., Dexheimer, S.L., Britt, R.D., Sauer, K. and Klein, M.P. (1990) The $S_0$ state of photosystem II induced by hydroxylamine: Differences between the structure of the manganese complex in the $S_0$ and $S_1$ states determined by x-ray absorption spectroscopy. *Biochem.*, **29**:4486.

Guiles, R.D., Zimmermann, J.L., McDermott, A.E., Yachandra, V.K., Cole, J.L., Dexheimer, S.L., Britt, R.D., Wieghardt, K., Bossek, U., Sauer, K. and Klein, M.P. (1990) The $S_1$ state of photosystem II: Differences between the structure of the manganese complex in the $S_2$ and $S_1$ states determined by x-ray absorption spectroscopy. *Biochem.*, **29**:471.

Holbrook, S. and Kim, S-H. (1989) "Molecular" model of the G protein a subunit based on the crystal structure of the c-H-*ras* protein. *Proc. Nat. Acad. Sci., USA*, **86**:1751-1755.

Holmes, K.T., Dyne, M., Williams, P.G., May, G.L., Tripp, E., Wright, L.C. and Mountford, C.E. (1989) Vinblastine sensitivity of leukaemic lymphoblasts modulated by serum lipid. *FEBS Lett.*, **244(1)**:103-107.

Hou, Y.M., Kim, R. and Kim, S-H. (1988) Expression of mouse metallothionein-I gene in *E. coli*: Increased tolerance to heavy metals. *Biochim. Biophys. Acta*, **951**:230-234.

Janes, S.M., Mu, D., Wemmer, D.E., Smith, A.J., Kaur, S., Maltby, D., Burlingame, A.L. and Klinman, J.P. (1990) A new redox cofactor in eukaryotic enzymes: Identification of 6-hydroxydopa at the active site of bovine serum amine oxidase. *Science*, **248**:981.

Jeffries, C.D., Lam, Q.H., Kim, Y., Kim, C.M., Zettl, A. and Klein, M.P. (1989) Nonlinear electrodynamics in the granular superconductor $YBa_2Cu_3O_7$: Experiments and interpretations. *Phys. Rev. B.*, **39**:11,526-11,537.

Kahn, J.D. and Hearst, J.E. (1989) The reversibility of nucleotide incorporation by *E. coli* RNA polymerase and its effect on fidelity. *J. Mol. Biol.*, **205**:291-314.

Kao, J.P.Y., Isaacs, P.T. and Hearst, J.E. (1990) The molecular and stereochemical structures of photoproducts generated by UV-irradiation of 4'-hydroxymethyl-4,5',8-trimethylpsoralen in aqueous solution. *Photochem. and Photobiol.*, **51**:273-283.

Keuper, H.J.K. and Sauer, K. (1989) Effect of photosystem II reaction center closure on nanosecond fluorescence relaxation kinetics. *Photosyn. Res.*, **20**:85-103.

Kim, S-H., de Vos, A., Tong, L., Milburn, M., Matias, P., Jancarik, J. and Nishimura, S. (1988) *Ras* oncogene proteins: Three-dimensional structures, functional implications, and a model for signal transduction. Cold Spring Harbor Symposium, **53.**

Kohwer, D., Taylor, S.E., Gordon, B.E., Otvos, J.W. and Calvin, M. (1989) Liquid fuels from mesua ferrea L seed oil. *J. Am. Oil Chem. Soc.*, **66**:223.

Kumar, N.V., Pease, J.H.B., Schweitz, H. and Wemmer, D.E. (1988) Solution structure of sea anemone toxins by NMR. *Am. Chem. Soc. Symp. Series*, "Natural Toxins from Marine and Aquatic Environments."

Maulbecker, C., Mohr, I., Gluzman, Y., Bartholomew, J. and Botchan, M.A. (1990) Deletion in the SV40 large T antigen impairs lytic replication in monkey cells *in vivo*, but enhances replication *in vitro*: A new complementation group in T antigen. *EMBO*, Cold Spring Harbor Conference on Eukaryotic DNA Replication, Cold Spring Harbor, NY.

Maxson, P., Sauer, K., Zhou, J., Bryant, D.A. and Glazer, A.N. (1989) Spectroscopic studies of cyanobacterial phycobilisomes lacking core polypeptides. *Biochim. Biophys. Acta.*, **977**:40-51.

McDermott, A.E., Yachandra, V.K., Guiles, R.D., Sauer, K. and Klein, M.P. (1989) EXAFS structural study of Fx, the low-potential Fe-S center in photosystem I. *Biochem.*, **28**:8056-8059.

Milburn, M.V., Tong, L., de Vos, A.M., Brünger, A., Yamaizumi, Z., Nishimura, S. and Kim, S-H. (1990) Molecular switch for signal transduction: Structural differences between active and inactive forms of protooncogenic *ras* proteins. *Science*, **247**:939-945.

Morimoto, H., Williams, P.G. and Saljoughian, M. (1989) Tritium labelling by thermally generated tritons. *Synthesis and Applications of Isotopically Labelled Compounds 1988 Proc. 3rd Int. Symp., Innsbruck).* (Baillie, T.A. and Jones, J.R., eds.) Elsevier, Amsterdam, p123-128.

Mukerji, I. and Sauer, K. (1989) Temperature-dependent steady-state and picosecond kinetic fluorescence measurements of a photosystem I preparation from spinach. In: *Photosynthesis* (W.R. Briggs, ed.) Alan R. Liss, Inc., NY, p105-122.

Mukerji, I. and Sauer, K. (1990) A spectroscopic study of a photosystem I antenna complex. In: *Current Research in Photosynthesis* (Baltsheffsky, M., ed.) Kluwer Academic Publs., Dordrecht, The Netherlands, **Vol II**:321-324.

Nakata, M. and Frei, H. (1989) Stereoselective photooxidation of trans 2-butene to epoxide by nitrogen dioxide with red light in a cryogenic matrix. *J. Am. Chem. Soc.*, **11**:5240-5247.

Nakata, M. and Frei, H. (1989) Stereocontrol of the red light induced photoepoxidation of 2-butene by nitrogen dioxide in solid Ar. *J. Phys. Chem.*, **93**:7670-7677.

Nakata, M. and Frei, H. (1989) Stereoselective photooxidation of butenes by electronically excited $NO_2$. *J. Chem. Soc.* Jpn., p1412-1417.

Newmark, R.D., Un, S., Williams, P.G., Carson, P.J., Warren, N., Morimoto, H. and Klein, M.P. (1990) $^1H$ nuclear magnetic resonance study of anaerobic glycolysis in packed red blood cells. *Proc. Natl. Acad. Sci., USA.*, **87**:583-587.

Pearlman, D.A. and Kim, S-H. (1990) Atomic charges for DNA constituents derived from single-crystal x-ray diffraction data. *J. Mol. Biol.*, **211**:171-187.

Pease, J.H.B., Kumar, N.V., Schweitz, H. and Wemmer, D.E. (1989) NMR Studies of toxin III from the sea anemone *Radianthus paumotensis* and comparison of its secondary structure with related toxins. *Biochem.*, **28**:2199.

Pelton, J.G. and Wemmer, D.E. Structural characterization of a 2:1 distamycin A - d(CGCAAATTGGC) complex by two-dimensional NMR. *Proc. Natl. Acad. Sci., U.S.A.*, in press.

Pelton, J.G. and Wemmer, D.E. (1989) Structure and dynamics of distamycin A with d(CGCAATTGGC) d(GCCAATTTGCG). *Frontiers of NMR in Molecular Biology, UCLA*, Alan R. Liss, Inc., NY, **Vol 109**.

Peng, C.T. (1989) Temperature and surfaces in tritium labeling. *Synthesis and Applications of Isotopically Labelled Compounds 1988 (Proc. 3rd Int. Symp., Innsbruck).* (Baillie, T.A. and Jones, J.R., eds.) Elsevier, Amsterdam, p525-528.

Pratum, T.K. and Klein, M.P. (1989) A $^{2}H$ and $^{14}N$ Study of molecular motion in polycrystalline choline chloride salts. *J. Mag. Res.*, **81**:350-370.

Puglisi J.D. and Tinoco, Jr., I. (1989) Absorbance melting curves of RNA. In: *RNA Processing Methods in Enzymology* (Dahlberg, J.E. and Abelson, J.N., eds.) Academic Press, NY, **180**:304-325.

Rine, J. and Kim, S-H. (1990) A role for isoprenoid lipids in the localization and function of an oncoprotein. *The New Biologist*, **3**:219-226.

Ross, W.S., Hardin, C.C., Tinoco, Jr., I., Rao, S.N., Pearlman, D.A. and Kollman, P.A. (1989) Effects of nucleotide bromination on the stabilities of Z-RNA and Z-DNA: A molecular mechanics and thermodynamic perturbation study. *Biopolymers*, **28**:1939-1957.

Saljoughian, M. (1990) Synthesis and applications of tritiated affinity probes. In: *Isotopes in the Physical and Biomedical Sciences, Labelled Compounds (Part A).* (Buncel, E. and Jones, J.R., eds.) Elsevier: Amsterdam, **Vol 2(2)**.

Saljoughian, M., Morimoto, H., Dorsky, A.M., Rapoport, H., Andres, H., Tang, Y.S. and Susan, A. (1989) A new and efficient synthesis of monotritiomethyl iodide. *J. Label. Compds. Radiopharm.*, **27(7)**:767-776.

Saljoughian, M., Morimoto, H. and Rapoport, H. (1989) Synthesis of monotritiomethyl iodide from thioethers. Hydrogenolysis in the presence of thioethers. *J. Org. Chem.*, **54(19)**:4689-4691.

Saljoughian, M., Morimoto, H. and Rapoport, H. (1989) A new route towards the synthesis of multi-tritiomethyl iodide. *Synthesis and Applications of Isotopically Labelled Compounds 1988 (Proc. 3rd Int. Symp., Innsbruck).* (Baillie, T.A. and Jones, J.R., eds.) Elsevier, Amsterdam, p517-520.

Saljoughian, M., Morimoto, H. and Williams, P.G. (1990) A general synthesis of high specific activity tritiomethyl iodide. *J. Chem. Soc., Perkin Trans.*, **2**, in press.

Salama, F. and Frei, H. (1989) Near infrared light induced reaction of singlet SO with allene and dimethylacetylene in a rare gas matrix. Infrared spectra of two novel episulfoxides. *J. Phys. Chem.*, **93**:1285-1292.

Sauer, K. (1990) Photosystem II and water oxidation. In: *Current Research in Photosynthesis* (Baltsheffsky, M., ed.) Kluwer Academic Publs., Dordrecht, The Netherlands, **Vol I**:675-684.

Sauer, K. and Klein, M.P. (1989) Photosystem II and oxygen evolution. In: *Current Research in Photosynthesis.* (Baltscheffsky, M., ed.) Kluwer, Amsterdam, **Vol I(3)**:675-684.

Scalettar, B.A., Hearst, J.E. and Klein, M.P. (1989) Fluorescence spectroscopic studies of self and mutual diffusion in entangled DNA solutions. *Macromolecules*, **22**:4550-4559.

Scalettar, B.A., Selvin, P.B., Axelrod, D., Hearst, J.E. and Klein, M.P. (1988) A fluorescence photo-bleaching study of the microsecond reorientational motions of DNA. *Biophysical J.*, **53**:215-226.

Schmidt, A., Sandmann, G., Armstrong, G.A., Hearst, J.E. and Boger, P. (1989) Immunological detection of phytoene desaturase in algae and higher plants using an antiserum raised against a bacterial fusion-gene construct. *Eur. J. Biochem.*, **184**:375-378.

Schultz, P.G., Hoyer, D. and Cho, H. (1990) A new strategy for the selective cleavage of proteins. *J. Am. Chem. Soc.*, **112**:3249.

Shi, Y.B., Lipson, S.E., Chi, D.Y., Spielmann, H.P., Monforte, J.A. and Hearst, J. E. (1990) Applications of psoralens as probes of nucleic acid structure and function. *Bioorganic Photochemistry: Photo-chemistry and the Nucleic Acids.* (Morrison, H., ed.) John Wiley & Sons, Inc., NY, **1**:341-378.

Spreer, L.O., Leone, A., Mailiyackel, A.C., Otvos, J.W. and Calvin, M. (1988) Chemistry of a high oxidation level manganese porphyrin in aqueous solution. *Inorg. Chem.*, **27**:2401.

Tinoco, Jr., I., Aboul-ela, F., Hardin, C.C., Puglisi, J.D., Varani, G., Walker, G.T., Wolk, S.K. and Wyatt, J.R. (1989) Nucleic acid structure from A to Z. In: *Frontiers of Macromolecular Science.* (Saegusa, T., Higashimura, T. and Abe, A., eds.) Blackwell Scientific Publications, Oxford, p519-524.

Tinoco, Jr., I., Puglisi, J.D. and Wyatt, J.R. (1990) RNA folding. In: *Nucleic Acids and Molecular Biology.* (Eckstein, F. and Lilley, D.M.J., eds.) Springer-Verlag, NY, p205-226.

Tong, L.A., de Vos, L., Milburn, M., Jancarik, J., Miura, K., Ohtsuka, E., Nogushi, S., Nishimura S. and Kim, S-H. Structural differences of transforming *ras* from the normal protein. *Nature*, **33:**90-93.

Un, S. and Klein, M.P. (1989) Study of $^{31}P$ chemical shift sensors and their correlation of molecular structure. *J. Am. Chem. Soc.*, **111**:5119-5124.

Varani, G.,Wimberly, B. and Tinoco, Jr., I. (1989) Conformation and dynamics of an RNA internal loop. *Biochem.*, **28**:7760-7772.

Williams, P.G. (1990) $^{3}H$ NMR studies of hydrogen isotope exchange reactions. In: *Isotopes in the Physical and Biomedical Sciences, Labelled Compounds (Part B).* (Buncel, E. and Jones, J.R., eds.) Elsevier, Amsterdam, **Vol 1(2),** in press.

Williams, P.G., Morimoto, H., Gehring, K.B., Nikaido, H., Carson, P.J., Un, S., Klein, M.P. and Wemmer, D.E. (1989) Following macromolecular interactions and sugar metabolism using site specific $^{3}H$ labelling and NMR spectroscopy. *Synthesis and Applications of Isotopically Labelled Compounds 1988 (Proc. Third Int. Symp., Innsbruck).* (Baillie, T.A. and Jones, J.R., eds.) Elsevier, Amsterdam, p487-492.

Williams, P.G., Morimoto, H., Saljoughian, M. and Andres, H. (1989) Advances in high specific activity tritide chemistry. *Trans. Am. Nucl. Soc.*, **60**:34-36.

Williams, P.G., Morimoto, H. and Saljoughian, M. (1989) The old and the new: Studies of metal-catalyzed exchange by $^{3}H$ NMR spectroscopy. *Synthesis and Applications of Isotopically Labelled Compounds 1988 (Proc. 3rd Int. Symp., Innsbruck).* (Baillie, T.A. and Jones, J.R., eds.) Elsevier, Amsterdam, p55-60.

Williams, P.G., Morimoto, H. and Wemmer, D.E. (1988) Application of modern $^{3}H$ NMR techniques to the analysis of complex isotopic products from a hydrogenation reaction. *J. Am. Chem. Soc.*, **110(24):**8038-8044.

Wolk, S., Thurmes, W.N., Ross, W.S., Hardin, C.C. and Tinoco, Jr., I. (1989) Conformational analysis of $dC_3G_3$: A B-family duplex in solution. *Biochem.*, **28**:2452-2459.

Wyatt, J.R., Puglisi, J.D. and Tinoco, Jr., I. (1989) Pseudoknotted RNA oligonucleotides. In: *Molecular Biology of RNA* (Cech, T., ed.) UCLA Symposia on Molecular and Cellular Biology, Alan R. Liss, Inc. NY, **94**:25-32.

Wyatt, J.R., Puglisi, J.D. and Tinoco, Jr., I. (1989) RNA folding: Pseudoknots, loops, and bulges. *Bioessays*, **11**:100-106.

Yachandra, V.K., Guiles, R.D., McDermott, A.E., Cole, J.L., DeRose, V.J., Zimmermann, J.L., Sauer, K. and Klein, M.P. (1988) X-ray absorption spectroscopy of Mn in the photosynthetic apparatus. XAFS V. *Proc. of the 5th International Conference on X-Ray Absorption Fine Structure* (deLeon, J.M., Stern, E.A., Sayers, D.E., Ma, Y. and Rehr, J.J., eds.) North Holland, Amsterdam, p78-80.

Yachandra, V.K., Guiles, R.D., McDermott, A.E., Cole, J.H., DeRose, V.J., Zimmermann, J.L., Sauer, K. and Klein, M.P. (1989) X-ray absorption spectroscopy of Mn in the photosynthetic apparatus. *Physica B.*, **158**:78-80.

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