LBL--27460

DE90 008061

Life Sciences

Lawrence Berkeley Laboratory 1988

Lawrence Berkeley Laboratory
One Cyclotron Road
Berkeley, California 94720

July 1989

This work was supported principally by the Office of Health and Environmental Research, Office of Energy Research, U.S. Department of Energy, under Contract DE-AC03-76SF00098. Additional support came from the Office of Basic Energy Sciences, Office of Energy Research, U.S. DOE; the National Institutes of Health, U.S. Department of Health and Human Services; the U.S. Department of Agriculture; the Armed Forces Radiobiology Research Institute; the National Aeronautics and Space Administration; the Whitaker Foundation; Liposome Technologies, Inc.; and the Monsanto Corporation.

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FOREWORD

LIFE SCIENCES RESEARCH AT LBL HAS BOTH a long history and a new visibility. The physics technologies pioneered in the days of Ernest O. Lawrence found almost immediate application in the medical research conducted by Ernest's brother, John Lawrence. And the tradition of nuclear medicine continues today, largely uninterrupted for more than 50 years. Until recently, though, life sciences research has been a secondary force at the Laboratory, in light of the Lab's position as an international leader in high-energy and nuclear physics. Today, in keeping with a decade-old vision for LBL, a true multiprogram laboratory has emerged, in which the life sciences participate as a full partner.

An important part of this change has been the growth of a multidisciplinary spirit—ensuring not only that the Laboratory pursues diverse research interests, but also that it pursues them with the full complement of tools and talents available here. Two examples, both described in more detail in the following pages, serve to illustrate this point. The LBL Human Genome Center is our contribution to the growing international effort to map, and eventually to sequence, the human genome. Its achievements during one short year of activity have been impressive—but more to the point, they represent the efforts of five LBL divisions, including Engineering, Materials and Chemical Sciences, and Information and Computing Sciences, along with Cell and Molecular Biology and Chemical Biodynamics. A second initiative, the ALS Life Sciences Center, also had its genesis during 1988, borne of the expressed needs of biomedical researchers for full experimental access to new synchrotron radiation facilities. The Center will thus comprise not only beamlines and experimental end stations, but also supporting laboratories and office space for scientists from across the U.S. And once again, this effort reflects a confluence of scientific disciplines—this time represented by individuals from the life sciences divisions and by engineers and physicists associated with the Advanced Light Source project.

And finally, this report itself, the first summarizing the efforts of all four life sciences divisions, suggests a new spirit of cooperation—cooperation achieved with no loss of diversity.

PAUL H. SILVERMAN
Associate Laboratory Director for Life Sciences
10 July 1989

Life Sciences Staff

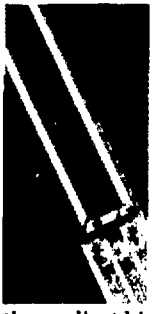
Paul H. Silverman, Associate Laboratory Director for Life Sciences

Igor R. Blake
Janice C. Demoor
Wendell Hom
Jody L. Jones
Mary Jordan
Sarah Ann Lee
Allan W. Long
Vera Martin
Laura E. Martinez
Louise Ray
Yolanda E. Reyna
Karen S. Ross
Dolores Ruff
Lori E. Ryan
Katherine A. Swensen
Frank T. Upham
Douglas Vaughan
Christine P. Villaluz
Mary L. Worth

Animal Care Facility

Robert W. Springsteen
Charles M. Allison
Gerald L. Brooks
Freddie L. Crenshaw, Jr.
Roscoe Frazier
Victor D. Smith

John H. Lawrence, Director Emeritus, Donner Laboratory



RESEARCH MEDICINE AND RADIATION BIOPHYSICS

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 had their effect, the focus of the division continues to be the investigation of human physiology and disease, using the tools afforded by nuclear physics. The use of radionuclides as therapeutic agents in the prewar years 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 the Bevalac. 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, now more than 40 years old. (During 1988 this work was conducted in the Cell and Molecular Biology Division; see pages 36–39.)

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, as well as providing a full complement of ancillary laboratory facilities for local and visiting scientists. The ALS will thus serve a well-established community of crystallographers with high fluxes of 10-keV 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 nm.

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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 in the service of medicine. This convergence of technological development and medical research is especially evident in our broad program of research on brain 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). 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 vivo. 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 diseases such as schizophrenia and affective disorders.

PET and NMR Studies

Positron-emission tomography is based on the use of metabolic tracers that contain a positron-emitting isotope. Decay of the isotope generates a positron, which then immediately annihilates with a nearby electron (its antiparticle) to produce two photons. The simultaneous detection of these photons, traveling in opposite directions, pinpoints the position of the original event. Different tracers can be chosen, or synthesized, to investigate different kinds of metabolic activity, and time-dependent studies can be carried out to assess the kinetics of metabolic processes. Many patient studies, for example, are done with ^{18}F-labeled fluorodeoxyglucose (FDG), a glucose analog that is taken up by actively metabolizing brain tissue. The highest-resolution instrument now available for such studies is the Donner 600-Crystal Positron-Emission Tomograph (Figure 1), commissioned in 1986 for studies of brain metabolism. The resolution of this system is 2.6 mm FWHM—at least twice as good as that of any other instrument. The value of this increase in resolution can be seen in Figure 1, where evidence of renewed tumor growth, visible in a high-resolution PET 600 image, cannot be seen in an image obtained with an older tomograph.

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 the newly developed radiopharmaceutical ^{122}I-labeled *N,N,N'*-trimethyl-*N'*(2-hydroxyl-3-methyl-5-iodobenzyl)-1,3-propanediamine (HIPDM), whose short half-life (210 seconds) makes repeated measurements possible during one patient sitting. Thus, we can measure unstimulated blood flow values, then, following some stimulus, record the stimulated flow.

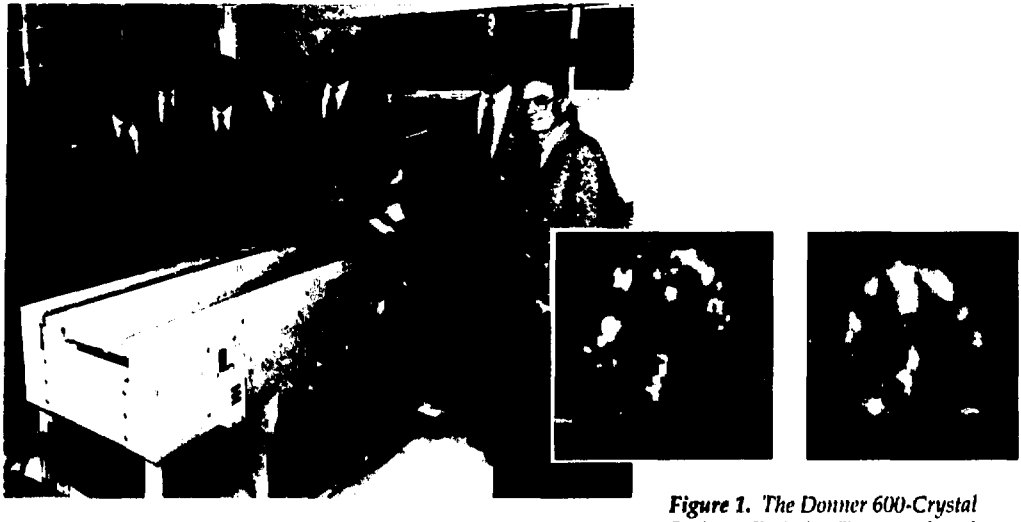


Figure 1. *The Donner 600-Crystal Positron-Emission Tomograph and a pair of PET images of the human brain, showing the improved resolution of this instrument, compared to that of a 280-crystal instrument. The tomograph is shown with members of the multi-divisional team responsible for its design, development, and construction. In both images, the light areas represent regions of active glucose metabolism, as measured by the uptake of an ^{18}F-labeled glucose analog. Both images show areas of low glucose uptake at the lower right, indicating necrotic tissue. The diagnosis of cerebral tumor, however, depends upon detection of a ring of high activity around the necrotic region. This ring is clearly seen only in the PET 600 image (on the left). [CBB 881-599 and XBB 884-2937]*

One such stimulus studied during 1988 was 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 10–15 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; 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, for example, the temporal cortex.

In other studies, PET and NMR can be 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 the process of brain necrosis in a dog exposed to a lethal dose of heavy-ion radiation. An understanding of this process is especially important in the context of the heavy-ion therapy program described on pages 8–10. Together, the use of PET and NMR allowed us to follow (i) changes in glucose metabolism in the

brain (PET with labeled FDG), (ii) changes in the permeability of the blood-brain barrier (PET with ^{82}Rb, which does not normally diffuse into the brain), and (iii) brain tissue edema and abnormal extracellular fluid characteristics that arise following radiation damage (proton NMR imaging).

Tracer Studies of Schizophrenia

A third type of study relied on neither PET nor NMR, using instead classical tracer techniques. In our work on schizophrenia and affective disorders, for example, we looked for changes in the methyl carbon pathway by injecting ^{11}C- or ^{14}C-labeled *S*-adenosyl methionine (SAM), 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 in turn that they may suffer an enzyme deficit in the methyl carbon pathway. This can affect both the activity of neurotransmitters and the normal functioning of the phospholipid membrane transport system. To pursue this investigation further, we plan to search the sequence of methyl-metabolism products for an enzymatic "bottleneck." 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 ^{14}C atoms, thus enhancing detection sensitivity by a thousandfold. Such an instrument, shown in Figure 2, would also have utility in studies of other metabolic diseases, of which more than 3000 are known, and in studies of the metabolism of drugs and toxic agents.

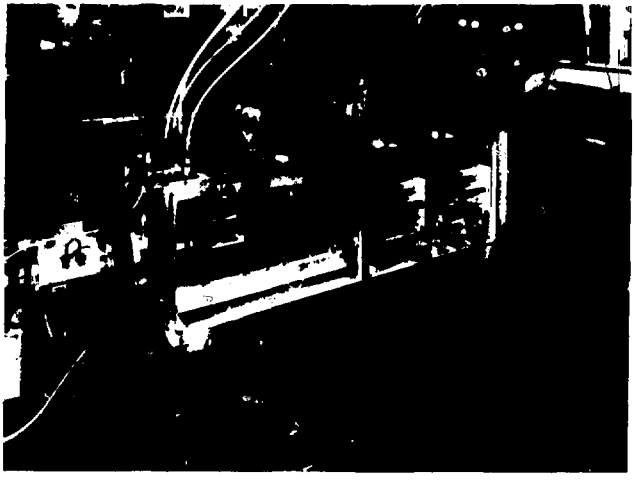


Figure 2. *A prototype biomedical "cyclotrino," conceived and constructed in LBL's Physics Division. A cyclotrino devoted to tracer studies would enhance detection sensitivity to isotopes such as ^{14}C by a thousandfold, thus making feasible a host of new studies of human metabolism. [CBB 888-8202]*

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 aimed at detecting vascular lesions in large blood vessels of rabbits. To do this, blood platelets were labeled with ^{68}Ga, which had been complexed to mercatopyridine-*N*-oxide. The labeled platelets were then injected intravenously into rabbits whose distal descending aortas had been scraped free of endothelial tissue 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 ^{68}Ga-platelets for imaging vascular lesions in the carotid arteries of human subjects. Efforts also began in 1988 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. During the past year, for example, our NMR group completed theoretical studies that provide a solid foundation for eventually conducting in vivo NMR at 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, however, is a more suitable scintillator than the bismuth germanate currently used. In 1988 we described a promising candidate material, cerium fluoride. We also designed and built a position-sensitive photodiode that improves the off-center resolution of PET images.

NMR Methods

Research applications of in vivo NMR spectroscopy are wide ranging, including studies of aging, atherosclerosis, ischemic 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. Improvements are possible by operating at higher magnetic fields, but higher fields typically require the application of radio-frequency 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 μsec) are applied to probe the "sample," followed by long relaxation times (about 1 sec) during which data are collected.

During the past year, we confirmed the feasibility, for in vivo studies, of a technique known as stochastic excitation, which circumvents the high-peak-power requirements of conventional pulsed NMR techniques. 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. In addition to our theoretical studies, experiments were carried out to compare alternative excitation schemes with regard to distortion and systematic noise.

In a second study aimed at broadening the utility of NMR spectroscopy, we sought to detect glycogen in vivo by ^{13}C-NMR spectroscopy, a technique that holds great promise for the in vivo study of biomolecules. ^{13}C-NMR is complicated by the large number of organic compounds in the body, whose individual resonances combine to produce a few broad lines; however, glycogen shows a resonance at 101 ppm, in an otherwise blank portion of the NMR spectrum. In spite of this, previous studies of glycogen utilization have relied on needle biopsies, which have shown that skeletal muscle contains roughly 4 mM glycogen. Figure 3 shows the first ^{13}C-NMR spectrum of glycogen in vivo, obtained from the natural abundance of ^{13}C. Owing to the success of this demonstration experiment, follow-up studies are under way that will look at carbohydrate metabolism by monitoring in vivo utilization of glycogen.

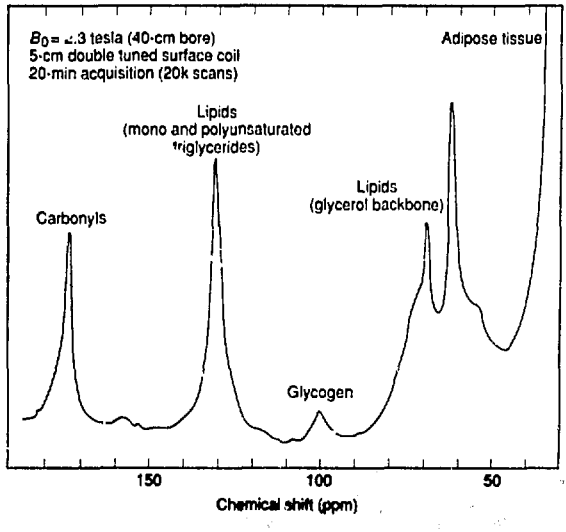


Figure 3. *A natural-abundance ^{13}C-NMR spectrum of human skeletal muscle (gastrocnemius). The relatively isolated position of the glycogen C-1 resonance made this successful in vivo spectrum possible. The concentration of glycogen in skeletal muscle is about 4 mM. [XBL 897-7658]*

Developments in PET Technology

Two especially notable developments characterized our efforts during 1988 to improve the state of current PET technology. The first was the discovery and characterization of a new scintillator, CeF_3. In a positron-emission tomograph, scintillators absorb the coincident x-rays 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, thus assuring adequate absorptivity; a short fluorescent decay lifetime, so that high count rates can be tolerated; and a reasonable light output. Cerium fluoride appears promising on all three counts. Its density is 6.16 g/cm^3, about 87% of that of bismuth germanate (BGO), which is today's standard scintillator, and its light output is about half that of BGO. Significantly, however, the fluorescence decay of CeF_3 can be described by a single exponential, with a time constant of 27 ± 1 nsec. For high-count-rate applications, this is much better than the 300-nsec decay time constant of BGO.

The second development was the fabrication and testing of a position-sensitive photodiode BGO detector. As shown in Figure 4, standard PET detector elements yield degraded resolution for "off-center" events, since one cannot tell where in the crystal the incident x-rays were detected. The new crystal design, also illustrated in Figure 4, combines a segmented silicon photodiode with a scintillator crystal in such a way that the distribution of fluorescence detected by the two photodiode segments reveals the approximate point at which the light originated.

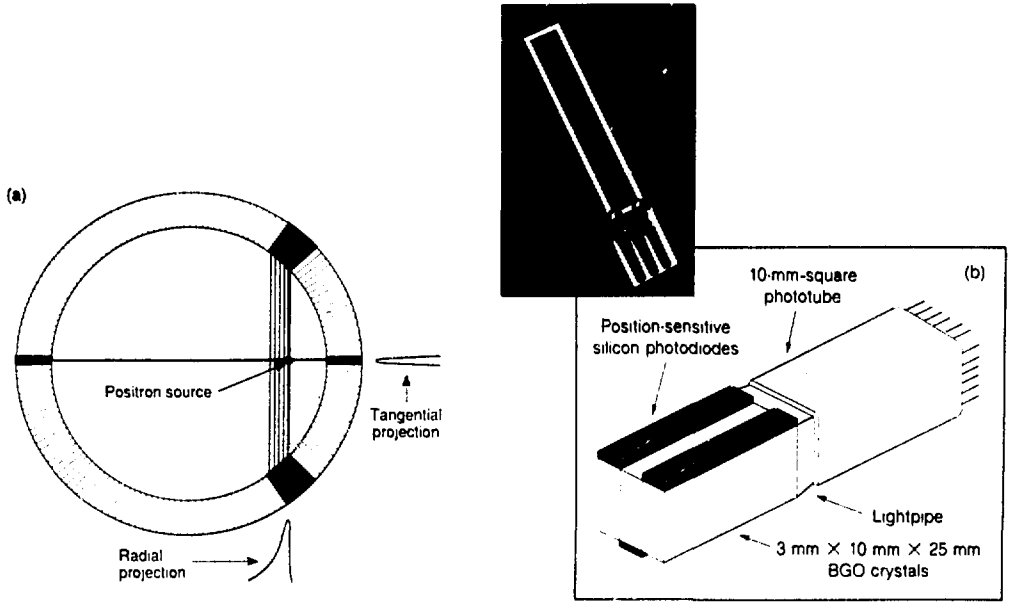


Figure 4. (a) A schematic illustration showing the cause of resolution degradation for off-center events in a conventional PET instrument. Each photon from the depicted off-center event typically follows a path that passes through more than one detector crystal. Since any one of these crystals can detect the photon, the position of its origin is obscured. Resolution is optimal in the tangential direction, worst in the radial direction. (b) A PET detector module incorporating a position-sensitive photodiode. The distribution of scintillation photons between the two triangular segments of the photodiode allows the position of the detection event to be localized along the length of the crystal. The location of off-center annihilations can thus be more accurately described. The inset shows a prototype photodiode. [XBL 897-7659, XBL 897-7660, and CBB 8810-10375]

In distinct contrast to x-rays, heavy ions and neutrons are especially effective in killing typically 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 manipulated in three dimensions, so that a well-defined volume of tissue is irradiated. As a consequence, the radiotherapy program at LBL has been able to demonstrate unequivocally that superior local cancer control can be achieved with heavy ions for a number of cancerous tumors. During 1988 our programs in this area proceeded on course, with a central aim being a continuing evaluation of different ion species with respect to their efficacy in treating different types of cancer. The heavy-ion radiotherapy trial accrued 64 patients during 1988, with an additional 28 patients receiving control irradiation. Nonclinical research proceeded in parallel, aimed mainly at understanding the physical and biological characteristics of heavy-ion beams and their effects in the brain.

Clinical Studies

Phase I and II studies have now been done using neon for several different anatomical regions and tumor types, including glioma of the brain, head and neck tumors, soft tissue and bone sarcoma, and tumors of the esophagus, pancreas, stomach, lung, and prostate. The results of these studies suggest further study of several cancers. Accordingly, we have begun Phase III trials for lung and prostate tumors, and randomized Phase II trials are planned for glioblastoma and base-of-skull tumors.

In our "dose localization studies," we take advantage of the biophysical characteristics of ion beams to deliver high doses to tumors adjoining critical anatomical structures. Through 1988, 105 patients had received heavy-ion irradiation for chordoma, chondrosarcoma, or meningioma of the base of the skull or the juxtospinal area. Tumor control within the irradiated area was observed in 72 (70%) of the patients, with a median follow-up of 27 months. Additional tumor sites and types also show long-term benefits from heavy-ion therapy. Among patients treated for lesions impinging on the brain stem and spinal cord, regardless of histology, local control has been achieved in 60% of the cases.

Other studies have focused on helium ions and were conducted at the 184-Inch Synchrocyclotron until its decommissioning in early 1988. During mid-1988, therefore, the ISAH patient positioner was relocated to a new biomedical radiation enclosure at the Bevalac. New beamline components were also fabricated, allowing flexible computer control of the diameter and residual range of the ion beam. The new beamline is shown

in Figure 5. Clinical research protocols resumed at the Bevalac in July 1988.

In one long-running helium-ion study, local control of uveal melanoma has been achieved in 97% of 208 patients, with a median follow-up of 54 months. Overall, 85% of the patients have retained their eyes, and more than half have post-treatment vision of 20/400 or better. The survival rate at 5 years is 80%. These are encouraging results, but it is possible that radioactive ^{125}I plaque therapy is equally good for some tumor locations and sizes. Thus, a randomized trial comparing helium therapy and ^{125}I plaque therapy is under way; it had accrued 142 patients by the end of 1988.

Also using helium ions, our stereotactic radiosurgery clinical program continued its studies on the use of focal irradiation to treat life-threatening intracranial vascular disorders. The ultimate aim is the transfer of safe, reliable, and reproducible treatment strategies to facilities based at community hospitals. Accordingly, the scope of the program is to optimize the dose, treatment volume, ion type, beam delivery technique, and treatment fractionation schedule for irradiation of intracranial arteriovenous malformations (AVMs) and neoplastic disorders. For several years, we have concentrated especially on AVMs, pathological clusters of blood vessels in the brain that are especially prone to hemorrhaging.

At the end of 1988, we were in the process of analyzing the conditions of treated patients who have been followed for two years or more. In a group of 65 patients for whom sequential cerebral angiograms were available, results show complete or partial cure in all cases. All small AVMs (those of 4 cm^3 or less) were completely obliterated, as were more than 80% of larger ones. 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 labeled FDG and HIPDM, as well as ^{82}Rb,

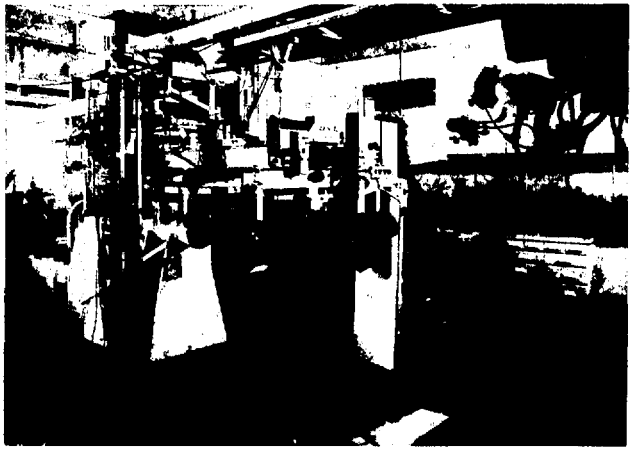


Figure 5. *The new biomedical beamline at the Bevalac. The new beamline, built to accommodate programs moved from the decommissioned 184-Inch Synchrocyclotron, includes a newly developed computer-controlled scatterer that comprises a variable-thickness water absorber and metal scattering plates. [CBB 887-7397]*

as PET tracers to assess changes in glucose metabolism, regional blood flow, and the integrity of the blood-brain barrier, and to correlate 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 26–27.) For example, a deficiency in current ion dose calculations is our inability to adequately account for multiple-scattering effects in complex media. We have therefore modified a so-called differential-pencil-beam (DPB) model for electron dose calculations, for application to the calculation of ion doses in heterogeneous media. Its success in preliminary studies with a simple phantom is illustrated in Figure 6, where the results of the modified DPB algorithm compared well with the results of a Monte Carlo simulation. The current algorithm, however, does not adequately account for the position of the heterogeneity (for example, the air cavity of the phantom in Figure 6), so further work is necessary. Nonetheless, prospects are encouraging that a more accurate dose-calculation model will ultimately be incorporated into the radiotherapy treatment-planning program.

Related studies include heavy-ion beam characterization and fragmentation studies; comparisons of physical 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.

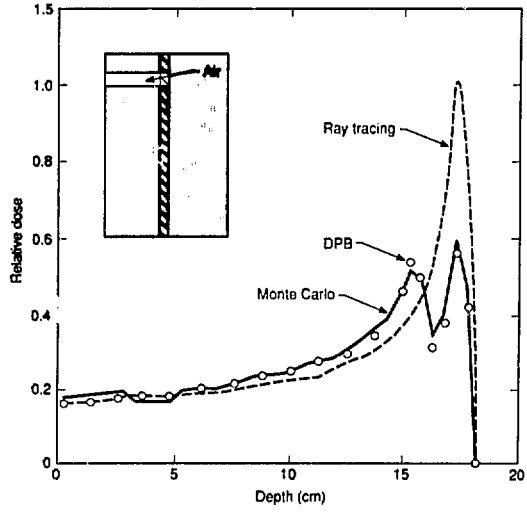


Figure 6. A plot of depth-dose distributions in the illustrated "phantom," calculated by ray tracing (dashed line), Monte Carlo simulation (solid line), and a modified differential-pencil-beam model (open circles). The success of the DPB model promises more-accurate dose calculations as part of radiotherapy treatment planning. [XBL 897-7661]

SPACE-RELATED RESEARCH AND OTHER STUDIES

Beyond the protection of the Earth's magnetic shield, cosmic ray particles consisting of energetic heavy nuclei—mainly the nuclei of such elements as helium, carbon, nitrogen, oxygen, and iron—present the major radiation hazard for manned spaceflight. On a 1000-day mission, protected by shielding typical of today's spacecraft, more than 10% of an astronaut's brain neurons would be hit by particles sufficiently energetic to cause some form of cell injury. A ground-based research program, pertinent to the NASA mission, has thus been under way at LBL for several years, centered around the heavy-ion beams available from the Bevalac. Along entirely different lines, our bioelectromagnetics research program continued in 1988, focusing on the effects of magnetic fields and of radio-frequency and microwave radiation on molecular, cellular, and tissue systems. The ultimate aim is to address health and safety issues related to emerging technologies that rely on high magnetic fields or on microwave and rf radiation.

Biophysics of Energetic Heavy Ions

To enhance our understanding of the interactions of cosmic rays with matter, we recently collaborated in a series of experiments with researchers from the NASA Langley Research Center in Hampton, Virginia. The central aim was to obtain comprehensive data on the interaction of a beam of 670-MeV/nucleon neon ions with a water column of different thicknesses. The electrical charge Z (and, in some cases, the isotopic mass) of primary beam particles and of the products of nuclear interactions emerging from the column were obtained for nuclei between beryllium (Z = 4) and neon (Z = 10). We also obtained the fluence of particles of a given charge, normalized to the incident beam intensity. Some of the key results are summarized in Figure 7.

In particular, projections of the data of Figure 7 onto the "floor" and "back wall" of the graph reveal pertinent aspects of heavy-ion interactions with matter. The dashed line on the floor depicts the rise in the average rate of linear energy transfer per ion as the incident beam approaches the stopping region. This curve is thus similar to the Bragg curve for pure neon. The solid circles on the back wall depict the exponential attenuation of the incident neon due to nuclear interactions in the water; however, this attenuation in the peak fluence at each depth is greater than the actual decrease of neon fluence, owing both to the broadening of each fluence spectrum and to changes in the detector acceptance as a function of depth. As part of this study, model calculations were also carried out, based on codes developed at LBL and at Langley, then compared with the data. Agreement was excellent in many respects, but the codes failed to accurately

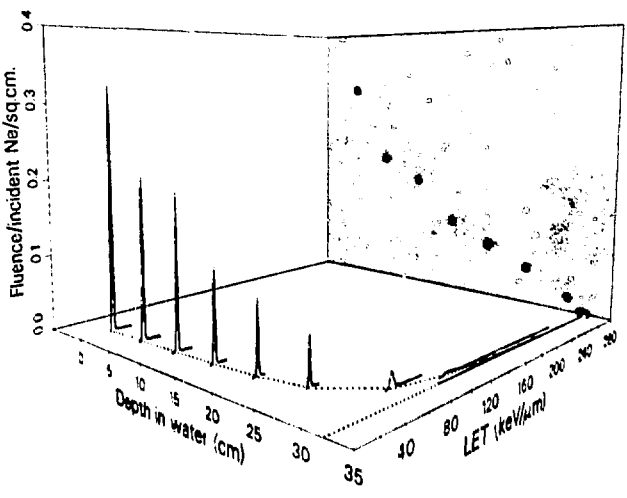


Figure 7. A plot showing the interplay of variables in a fragmentation experiment at the Bevalac, in which a 670-MeV/nucleon neon beam impinged on a water column. At each depth, the distribution of linear energy transfer has been plotted. The projection of the LET peaks is shown as a dashed line on the "floor" of the plot; it is similar to the Bragg curve for pure neon. The projection of the peak intensity onto the "back wall" indicates the exponential attenuation of the beam. [XBL 888-2761]

predict the distribution of secondary nuclei as a function of depth. As a consequence, we are currently developing more sophisticated transport codes, which will lead in turn to a more refined ability to predict the composition of radiation fields in matter.

Bioelectromagnetics Research

Several of the activities in the bioelectromagnetics research group focus on novel applications of electromagnetic fields to problems in the life sciences. One particularly promising effort was directed toward the use of pulsed electric and magnetic fields during liquid chromatography, to enhance the separation of large biopolymers. We have, for example, separated DNA molecules of fewer than 50,000 base pairs, which remain unseparated in the absence of pulsed fields. We have also used dc magnetic fields and microwave radiation to induce drug-loaded liposome vesicles to act as "molecular syringes." In one experiment, for example, the liposomes could be seen to bind selectively to the cell surfaces of target lymphocytes, then, when induced to do so by microwave radiation, to deliver a yellow fluorescent dye to the blood cells. This technique holds great promise for controlled drug delivery.

During the past year, we also completed construction of a 9-T magnetic circular dichroism (MCD) spectroscopy system. Studies are under way to characterize the MCD spectra of living cells after exposure to ionizing radiation. In Figure 8, a band at 315 nm, perhaps associated with histone-chromatid topology, shows significant sensitivity to such radiation.

In a final area of study, we are exploring the suggestion that exposure to steady magnetic fields may produce magneto-

chemical effects or electromagnetic effects in living systems. We have, for example, looked at ion current across axonal membranes in the sciatic nerves of frogs, during and after a 4-hour exposure to 7.5-T magnetic fields. Physical models predict possible changes in the conduction of nerve impulses, but our studies detected no changes in conduction velocity or action potential amplitude.

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 photons. By relying on special periodic magnetic arrays known as "wigglers" and "undulators," and by keeping its circulating electrons within the bounds of a narrow pencillike 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 crystallography. To exploit this resource for biology, we have therefore proposed an ALS Life Sciences Center, whose scope includes construction of two insertion devices (one wiggler and one undulator); an undulator beamline equipped for microimaging and microholography; a wiggler beamline equipped for diffraction and spectroscopy; and 11,100 square feet of fully equipped support laboratory and office space. The estimated cost of the project is $25.4 million; it is scheduled to commence in fiscal 1991 and to be completed in fiscal 1993.

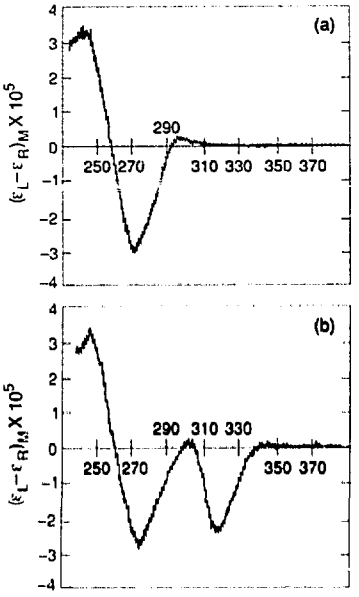


***Figure 8.** Magnetic circular dichroism spectra of hamster embryo cells, before (a) and after (b) exposure to ionizing radiation. The band at 315 nm is a protein signal and may be associated with histone-chromatid topology. [XBL 897-7662]*

Background of the Proposal

For more than two decades, lasers have dominated the visible and infrared regions of the electromagnetic spectrum. During this same period, the most powerful sources of x-ray and ultraviolet radiation (collectively, the XUV region) have been synchrotron light sources, where electrons circulating in an accelerator at relativistic velocities emit light as a by-product of their motion. Missing so far in the XUV has been light with many of the useful properties that are associated with the laser.

In essence, the ALS (see Figure 9), 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. Following its comple-

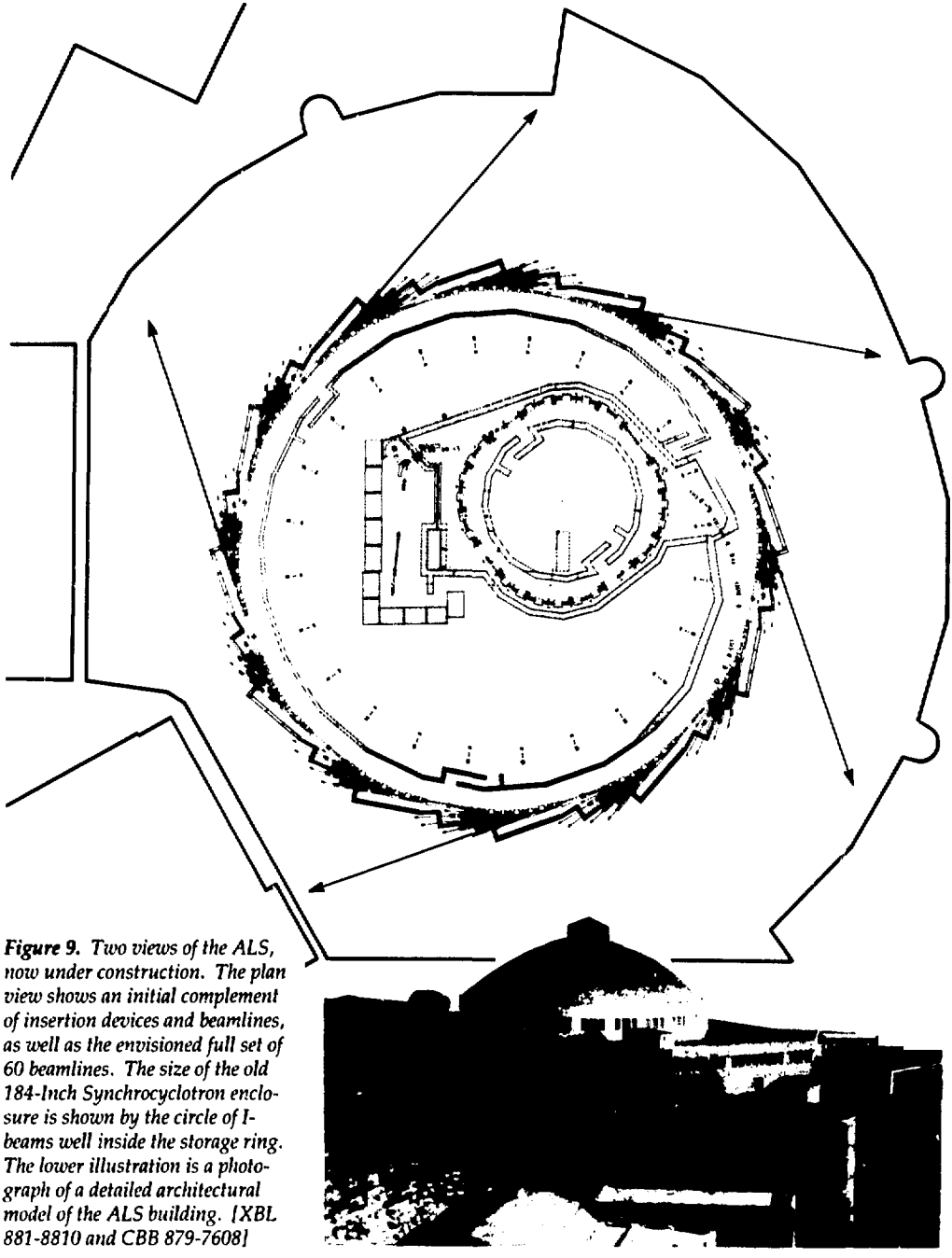


Figure 9. *Two views of the ALS, now under construction. The plan view shows an initial complement of insertion devices and beamlines, as well as the envisioned full set of 60 beamlines. The size of the old 184-Inch Synchrocyclotron enclosure is shown by the circle of I-beams well inside the storage ring. The lower illustration is a photograph of a detailed architectural model of the ALS building. [XBL 881-8810 and CBB 879-7608]*

tion in the spring of 1993, biologists can look forward to a host of new possibilities for the life sciences, including unprecedented microscopic views of cells and their substructures in their natural state, insights into multimolecular aggregates of bioorganic 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, it 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.

This proposal was catalyzed during 1988 by the Workshop on Life Sciences at the ALS, held in February at the University of California at San Francisco, and by subsequent meetings that further defined the needs of research biologists. In particular, it became clear that fully adequate facilities for life sciences research were not available at any operating synchrotron light facility. In response, a conceptual design proposal was prepared for the Life Sciences Center, as a basis for discussion within the life sciences community. By early 1989, a consensus had emerged that the Center should be included in the first phase of a major structural biology facilities initiative proposed by the DOE's Office of Health and Environmental Research, and in February 1989, a full conceptual design report for the Center was complete.

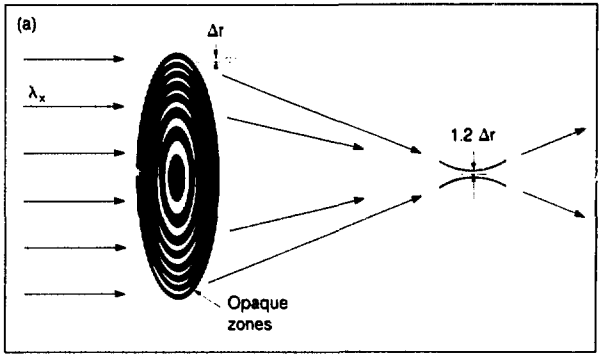
Research at the Life Sciences Center

The scientific case for a center for life sciences research at the ALS ultimately rests with the rough equivalence between the size of the feature one hopes to resolve and the wavelength of the probing light. Because the wavelength of x-rays is far shorter than that of visible light, far smaller features can, in principle, be discerned. If this theoretical equivalence can 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. In addition, beyond this relationship between resolution and wavelength, x-rays offer other features that make them particularly valuable as probes of biological matter.

Accordingly, the Center's scientific goals include, primarily, (i) new applications of x-ray methods already established and widely used, such as protein crystallography; (ii) full realization of the promise of newly emerging synchrotron-based microimaging methods, such as x-ray microscopy and holography; and (iii) assessing the feasibility of new technical approaches, such as merging spectroscopy and spatially resolved x-ray microimaging, and the development of novel means of extending imaging resolution toward theoretical limits.

The greatest 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 its natural state at resolutions never before achieved (Figure 10), and resolution may approach 10 nm by the time the ALS is commissioned. Imaging with soft x-rays—x-rays with wavelengths between roughly 1 and 5 nm—can be "natural" because (i) such radiation can penetrate objects several micrometers thick, and thus sectioning can be avoided; (ii) the object can be viewed while suspended in water at atmospheric pressure and body temperature; and (iii) water is transparent in the soft x-ray "water window" (between 2.3 and 4.4 nm), whereas various elements normally present in biological material, particularly carbon and nitrogen, absorb x-rays at the same wavelengths. This last virtue allows for contrast based on spatially resolved variations in the concentrations of natural substances, rather than by adding foreign ones.

In addition, the presence of discrete absorption edges for elements of biological interest in the x-ray domain opens the door to the possibility of elemental mapping, a technique that would exploit differences in elemental absorption at different wavelengths. Not only will it be possible to measure quantitatively the presence of particular elements in a spatially resolved



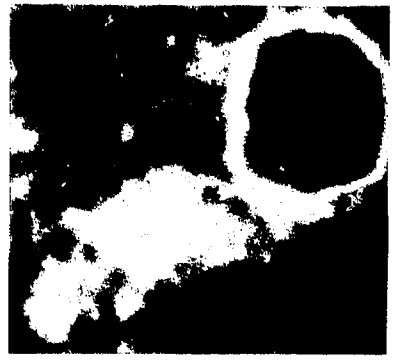


Figure 10. *(a) A schematic diagram of a Fresnel zone plate. Diffraction from the variably spaced rings focuses a plane wave to a small spot whose radius is 1.2 times the width of the outer zone. In a collaborative effort between LBL and IBM, zone plates have now been fabricated with outer zone widths of 40 nm. (b) An x-ray micrograph of a zymogen granule (a subcellular secretory structure extracted from the pancreas of a rat), obtained at a wavelength of 3.2 nm. 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. The transmitted x-ray intensity can be used directly to estimate protein content. [XBL 842-9405D and CBB 881-203E]*

fashion, but also organic chemical content can often be usefully determined from elemental information. More generally, if combined with spectroscopy, this method has the potential to provide a variety of direct information regarding the chemical form and reactive state of specific bioorganic molecules within the cell, as well as within isolated substructures.

X-ray spectroscopy has an excellent record of achievement in several areas of biological, biochemical, and biophysical research, and the parameters of the ALS offer exciting new opportunities in this area as well. Central to x-ray spectroscopy is the ability to associate spectral features with particular elements, or even elemental states. This is generally done by examining spectral features near particular elemental absorption edges or by means of fluorescent emission. The extra capabilities that the ALS will bring to such methods as EXAFS and XANES include the investigation of very dilute materials and the analysis of material that is spatially localized within some particular sample feature—a combination of microimaging and spectroscopy.

The ALS will provide photons in two energy regions that will be at the forefront of innovative biological spectroscopy. The first regime is that from 2 to 4 keV, which contains the *K*-edges of the elements phosphorus, sulfur, chlorine, potassium, and calcium, all of great importance and interest in biology. The second energy regime of particular interest is between 0.3 and 1.2 keV, where one finds the *L*-edges of the first series of transition elements, almost all of which are biologically important, as well as the *K*-edges of important light elements. It is in this range that the undulators planned for the ALS offer the greatest promise for high brightness, and the ability to carry out spatially resolved spectroscopy for elements in this region would be of important potential value.

A final area of promise at the ALS is x-ray diffraction and scattering. Most of the detailed 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 c-H-*ras* oncogene protein, described on pages 58–59.

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 (0.15 nm) required for the satisfactory resolution of macromolecules. Indeed, careful comparisons have been carried out between the ALS W13.6 wiggler and two established crystallography sources: the A1 wiggler at the Cornell synchrotron radiation facility and the Beamline 7.1 wiggler at SSRL. At a 10-keV photon energy, all three of these

sources produce essentially the same flux; however, the brightness of the ALS source is significantly better (theoretically, as much as 100-fold better) than the other two. (Recently commissioned sources at these facilities are brighter than the older sources, though still not as bright as the ALS wiggler is expected to be. Their fluxes are roughly comparable to those of the sources used in the comparison.) For some experiments, flux is the more important parameter of merit, but in others—for example, a measurement on a small crystal with a large unit cell (say, a virus)—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 regime to the characteristic size of various biological forms 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.

Research Medicine and Radiation Biophysics Division Staff

Thomas F. Budinger, Division Director
Stephen E. Derenzo, Deputy Division Director for Scientific Operations
Yukio Yano, Deputy Division Director for Administration

Faculty Scientists

Thomas F. Budinger[a,b]
Shirley N. Ebbe[b]
Jacob I. Fabrikant[b]
Martin H. Graham[a]
Yoshio Hosbuchi[b]
William J. Jagust[c]
Ronald M. Krauss[b]
Alex V. Nichols[a]
Jerome R. Singer[a]

Faculty Scientists (Guests)

Joseph R. Castro[b]
Theodore L. Phillips[b]

Staff Scientists

E. John Ainsworth
Melissa A. Austin
S. Jacob Bastacky
Kathleen M. Brennan
William T. Chu
J. Michael Collier
Pamela G. Coxson
Kanu B. Dalal
Stephen E. Derenzo
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Kenneth A. Frankel
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Mark H. Phillips
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James. B. Schmidt
Scott E. Taylor
Thomas S. Tenforde
Peter E. Valk
Karen M. Vranizan
Paul T. Williams
Mervyn Wong
Yukio Yano

Staff Scientists (Guests)

William F. Bennett
Eugene V. Benton[d]
Christopher Cullander
Mary M. Decker
John F. Dicello
Barry L. Engelstad
Edwin G. Garcia
Grant E. Gauger
Michael Goitein[d]
Yoshifumi Higashi
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Hiroo Ikehira
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Eva M. Salmeron
J. Philip Seab
Alexander T. Shulgin[d]
Argiroula A. Tzika
Lynn J. Verhey
Deborah C. Yager

Trainees (Postdoctoral Fellows and Graduate Students)

Stephen Bicknese
Blaise D. Frederick
John R. Keltner
Mark McCall
William W. Moses
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Technical Support Staff

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Sam T.S. Wong

Technical Support Staff (Guests)

Elizabeth A. Alonzo
John C. Bischof
Charlotte Brown
Marica Chen
Dennis Chin
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Luisa M. Disini
Maria K. Dybbro
Matthew N. Enmark
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Eunji Lee
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John T. Newland
Laura M. Palanck
Gabriel C. Paulson
Eva A. Penzes

[a]UC Berkeley
[b]UC San Francisco
[c]UC Davis
[d]Consultant

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Roberta Shew
Tom Tjoa
Greg Y. Uramoto
Troy E. Wilson

Administrative Support

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Diana E. Duhnke
Marybeth Gonzales

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CELL AND MOLECULAR BIOLOGY

IN 1987 AN LBL TASK FORCE REPORTED its findings with regard to the organization of life sciences research at the Laboratory. Among its conclusions was the view that providing "aggressive programmatic leadership within the area of cell and molecular biology . . . [is] the single most urgent issue that should be addressed. . . . The need to provide such leadership . . . leads naturally to the conclusion that cell and molecular biology should in the future be organized under its own separate administrative entity." This recommendation both recognized the excellence of ongoing research at LBL and argued for its expansion.

Accordingly, in 1988 the Cell and Molecular Biology Division was established to pursue multidisciplinary basic research in the broad areas of gene expression and molecular genetics. Its activities encompass a wide range of research topics, from studies of macromolecular structure by electron crystallography to research on mechanisms of carcinogenesis and the use of the Bevalac to study basic radiation biology. All of the efforts, however, are united by their ultimate focus on understanding how an organism's genomic program is expressed and regulated, how it maintains itself, how it is affected by radiation and toxic substances, and how it goes wrong in tumorigenesis.

The Cell and Molecular Biology Division is also the administrative home of the Human Genome Center, one of two DOE-designated centers for research aimed at ultimately mapping and sequencing the human genome. In line with the DOE's strategy for developing advanced tools for the effort before undertaking large-scale mapping or sequencing programs, the Center is the focus of related activities in several LBL divisions, including Engineering, Materials and Chemical Sciences, and Information and Computing Sciences, as well as the 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.

". . . all efforts focus on learning how an organism's genomic program is expressed and regulated, how it maintains itself, how it is affected by radiation and toxic substances, and how it goes wrong in tumorigenesis. . . ."

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 8–10). At the same time, they are closely tied to our studies of carcinogenesis and DNA repair. As it has for a decade and a half, much of the interest at LBL remains fixed on heavy ions, which, compared with x-rays, transfer large amounts of energy to the surrounding medium over very short distances at the end of their paths. Ongoing research extends from efforts to better characterize the physics of this energy deposition to experimental and theoretical attempts to understand the processes by which it can lead to carcinogenic transformation.

Radiological Physics

As a result of our work through 1988, we now have a reasonable understanding of the microscopic pattern of average energy deposition in media such a water, as well as the radiation chemistry that follows. As a result, we believe that characterizing energy deposition in volumes with nanometer dimensions is critical for understanding radiation-induced DNA damage. Our present studies thus focus on evaluating the magnitude of energy deposition at various "sites," such as sugars and bases, for different kinds of radiation. Using the existing estimate for the oscillator strength distribution, which is poorly known, we have made significant progress in characterizing ionization events at sugar sites, together with the subsequent strand breaks. We have also made plans to measure the oscillator strength distribution, using the Advanced Light Source, to increase our confidence in this important physical parameter.

Cellular and Molecular Radiobiology: Effects of Heavy Ions

In seeking to understand the effects of 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 chromatin breaks and rejoining, and evaluation of neon-ion–induced rearrangements in a single human chromosome. The second of these studies aims to uncover the mechanistic basis for the well-established observation that the effectiveness of radiation in killing cells depends greatly upon its LET value. The so-called relative biological effectiveness rises to a broad peak in the range of 100–200 keV/μm, then steadily decreases. If we postulate that unrepaired or misrepaired chromatin breaks are the only lesions 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 chromatin breaks in individual cells. The results illustrated in Figure 11, for example, show that the kinetics and the extent of chromatin-break rejoining depend on the LET of the radiation causing the breaks.

Radiation-Induced Cell Mutation and Neoplastic 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 genetic loci, TK and HPRT. How do the relative "yields" of these mutations depend on the density of energy deposition and how do the mutational mechanisms differ? A second effort centers around some of the events that accompany cell transformation: (i) chromosomal alterations in the vicinity of known proto-oncogenes, probed by means of in situ hybridization techniques; (ii) the appearance of restriction-fragment-length polymorphisms in the same region; and (iii) changes in gene expression, as measured by changes in RNA levels. An early result of this second project is the finding that two primary ionization events within 80 Å in DNA appears to cause oncogenic cell transformation. Finally, in a third area of interest, we are looking at tumorigenesis in several systems, with the aim of relating relative biological effectiveness to particle LET, track structure, and other physical parameters.

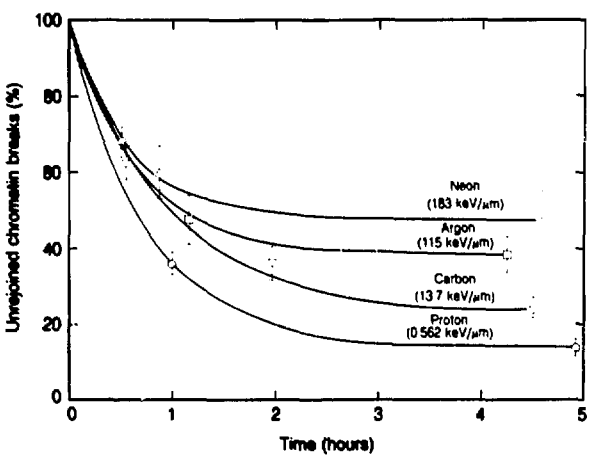


Figure 11. *A plot showing the time course of chromatin-break rejoining in CHO-tsH1 cells, after exposure to four types of radiation. Each curve shows a fast initial phase of damage repair, followed by a slower phase. Both the kinetics and the extent of break rejoining depend on the value of linear energy transfer for the damaging radiation. [XCG 884-6604A]*

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 normal to 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 (see Figure 12). Unrepaired single base-pair substitutions, for example, are among several mechanisms apparently capable of "turning on" oncogenes, and individuals with genetic-repair-deficient diseases are extremely cancer-prone. Our activities in the area of DNA damage and repair include research on how structural constraints and functional organization of the cell's genome affect repair processes, cloning of human DNA repair genes by complementation of the defect in human repair-deficient cells, studies of inducible responses to DNA damage, and in vitro studies of replication of damaged DNA templates. Significant effort also centers around research with yeast: We devote significant effort to understanding recombination repair and mitotic recombination in Saccharomyces cerevisiae, *we maintain a unique yeast stock center as an international resource, and we regularly update our widely used compilation of yeast genetic mapping data.*

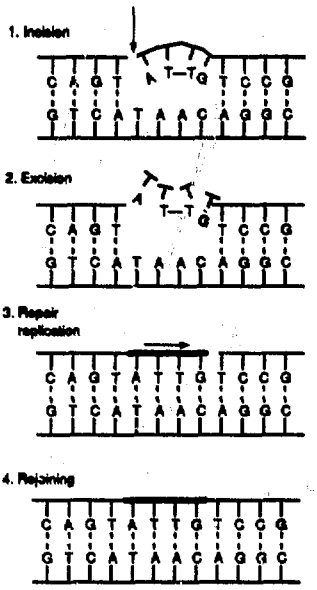


Figure 12. *A schematic depiction of a mechanism for repair of DNA damage. The section around a thymine dimer, a common product of damage caused by ultraviolet radiation, is first excised, then repaired by using the intact complementary strand as a template.* [XBL 897-7663]

Constraints on the Processing of DNA Damage

Several projects in the area of DNA damage and repair focus on how DNA sequence, replication forks, and functional genomic organization affect the processing of DNA damage. The mutagenic or lethal consequences of any particular lesion in DNA depend on cellular processing of that lesion, often by responses that are induced by the damage itself. A damage-inducible repair process in *E. coli*—long-patch excision repair—has been shown to be of general importance in a cell's resistance to lethal and replication-blocking effects of DNA damage. Results from a variety of experiments in our labs have suggested that the inducible process is required for repair of lesions near DNA replication forks, where structural constraints might hinder normal, or "constitutive," excision repair. We recently developed a two-dimensional agarose gel electrophoresis technique for resolving DNA fragments containing replication forks from linear DNA fragments, and we have now used it to obtain evidence that lesions in the vicinity of replication forks require the induced long-patch process for their repair.

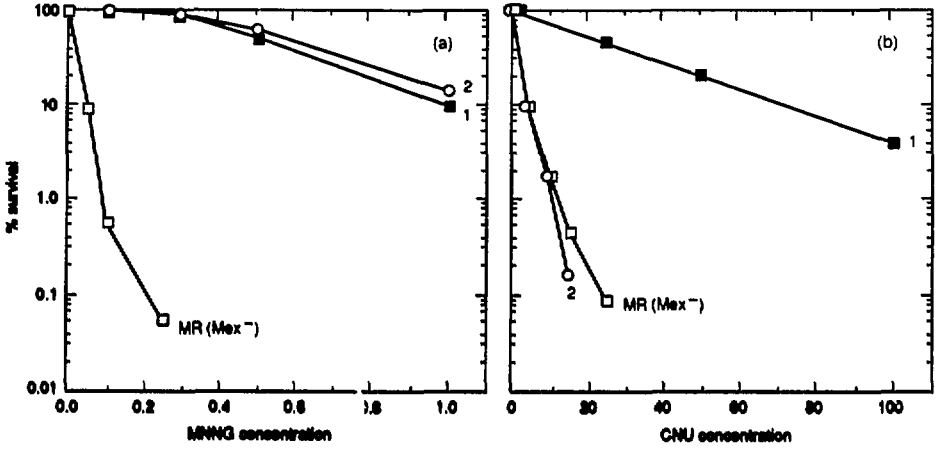
We are now attempting to understand the mechanism by which the induced process deals with the structural constraints

imposed by the blocked replication forks and to identify the induced proteins that are required. Various considerations suggest that lesions subject to long-patch repair might be those immediately behind the fork, and we are directing several approaches toward determining whether the long patches are preferentially located on the leading or the lagging strand. During the past year, we established a requirement for the direct participation of *recA* protein, in addition to some other as-yet-unidentified protein. In one model of the repair process, lesions introduced into parental DNA on the side of the lagging strand are unrepairable constitutively because of their single-stranded configuration, but the *recA* protein functions to place the partially replicated parental strands in alignment with each other, essentially by D-loop formation, so that the intact strand can function as a template for repair synthesis on the damaged strand. Future directions include the testing of this and other models. In addition, we are currently applying our two-dimensional gel technique to test whether the variant form of the human genetic-repair-deficient disease xeroderma pigmentosum is defective in the processing of lesions in the vicinity of replication forks.

Inducible Responses to DNA Damage

Another area of interest is the inducible responses, including enhanced tolerance, that can occur in cells following exposure to DNA-damaging agents. The long-term goal of our work with alkylating agents, for example, is to define the biological effect of specific carcinogen-DNA adducts and the role of particular cellular responses in overcoming these defects.

One DNA adduct formed by alkylating carcinogens, which seems to be primarily responsible for the mutagenic and carcinogenic effects of these agents is O^6-methylguanine (O^6MeG). Fast removal of such an adduct should be particularly important for the cell, and many cells, denoted Mex^+, do indeed have a special repair system for this lesion. Cells that do not repair O^6MeG are called Mex^-, and they are typically more sensitive than Mex^+ cells to the cytotoxic effects of monofunctional alkylating agents such as *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) and bifunctional alkylating agents such as chloroethylnitrosourea (CNU). Using a human cell line named MR, which has the Mex^- phenotype, we found that the phenotype of these cells can be permanently altered in two different ways by treating them with a highly toxic dose of an alkylating agent: (i) Cells surviving multiple doses of CNU become resistant to CNU by reverting to the Mex^+ phenotype; (ii) most cells surviving a highly toxic dose of MNNG become resistant to MNNG, even though they are still deficient in repair of O^6MeG, that is, they are still Mex^-. These two responses are illustrated in Figure 13. This finding suggests that the Mex^- phenotype is due to a regulatory change in the cells, so that the gene coding for the O^6MeG repair protein is not expressed.



To seek the cause of MNNG resistance in the resistant Mex⁻ variants, we isolated a number of cell hybrids combining sensitive and resistant Mex⁻ cells or combining sensitive Mex⁻ and Mex⁺ revertants. Survival of these hybrids after MNNG treatment showed that Mex⁺ resistance is dominant, whereas MNNG resistance of Mex⁻ cells is recessive. The different regulation of MNNG resistance of Mex⁺ and resistant Mex⁻ cells indicates a different biochemical basis. In a collaboration with Jeffrey Baker of UC Berkeley, we also determined intracellular levels of adenyldinucleotides (the so-called alarmones) after MNNG treatment. Increased levels were found only in resistant cells. However, an increased alarmone level alone is not sufficient for MNNG resistance, but rather seems to indicate a different means of processing alkylation damage in MNNG-resistant cells. We will pursue the characterization of these resistant variants by attempting to isolate damage-induced transcripts by hybridization subtraction of mRNA from untreated cells to cDNA from MNNG-treated cells. Transcripts that are increased in response to MNNG will be cloned into vectors and further characterized.

Figure 13. *Plots showing the response of human MR cells to N-methyl-N'-nitro-N-nitrosoguanidine (a) and chloroethylnitrosourea (b). Reversion of sensitive cells to a resistant phenotype can occur by two mechanism, one (curves labeled 1) that restores the ability to repair the damage caused by alkylating agents and a second (curves labeled 2) that leads to tolerance of MNNG but not CNU. [XBL 897-7664]*

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. 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. The most important has been to culture human and murine mammary epithelial cells as systems for the study of normal growth, differentiation, and transformation. In particular, we are interested in the role of cell-cell and cell-matrix interactions in gene expression and differentiation in these systems. Another approach to the same broad issue of differentiation and oncogenesis 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 hemopoesis—the differentiation of blood cells from a pluripotent precursor—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 capable of long-term growth, which has the characteristics of a stem cell population: Its phenotype can be modulated in culture by age, medium components, and the nature of the substratum. With this common resource now available, significant new findings are emerging from a number of laboratories, including insights into the normal mechanisms that control 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 protooncogenes in cell proliferation and on the interaction of epithelial cells with the procarcinogen benzo[a]pyrene (BaP).

We previously showed that human mammary epithelial cells readily convert BaP to its ultimate carcinogenic form, BPDE (benzo[a]pyrene-7,8-dihydroxy-9,10-epoxide), whereas fibroblast cells from the same tissue are much less capable of this reaction. More recently, we identified a previously uncharacterized BaP metabolite as 7-hydroxy benzo[a]pyrene and found that it is an extremely potent and selective inhibitor of the conversion of BP-diol to BPDE—the key step in the activation process. It thus appears that the activation of BaP in human mammary epithelial 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 and mesenchymal stroma. Interaction between these two tissue components is crucial to proper development and morphogenesis, 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 tissue from the mouse, we have looked at the role of the extracellular matrix in cell differentiation, and we have studied the role of epithelial cell polarity.

Among our findings, we showed that the extracellular matrix (ECM) shares with hormones the responsibility for regulating histogenesis and gene expression. Floating collagen gels, glutaraldehyde-fixed reconstituted basement membranes (so-called EHS matrixes), and unfixed EHS matrixes induce production of high levels of transferrin and caseins, but only the unfixed EHS matrix allows expression of whey acidic protein. We also demonstrated a dramatic effect of the ECM on the architecture of the epithelial cells in culture. On the EHS basement membrane, as shown in Figure 14, disaggregated cells form alveoluslike structures remarkably similar to physiological structures.

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 answer to how viruses cause cancer must come from studies in vivo. We are therefore using a replication-defective avian Rous sarcoma virus to study expression and inhibition of tumorigenesis in chick embryos. One dramatic result of the past year was the finding that the embryonic environment of the chick suppresses the oncogenic potential of the viral gene. After

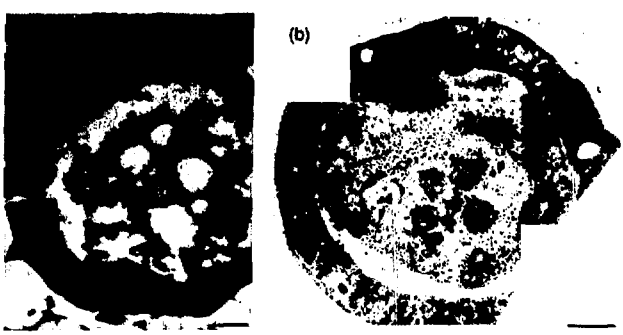


Figure 14. *(a) A photomicrograph of a sectioned alveolus from a lactating murine mammary gland. (b) A photomicrograph of the structure formed by disaggregated cells grown on a reconstituted basement membrane (extracellular matrix, or ECM) obtained from a murine tumor that overproduces ECM. The reaggregated cells secrete milk proteins into the central lumin. Thus, the ECM allows cells to recapitulate both morphological and functional differentiation. [CBB 897-5601*

4-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 related studies with newborn chicks, we have also found that wounding plays a role as a co-carcinogen in tumorigenesis following injection with the Rous sarcoma virus.

Hemopoiesis

Hemopoiesis—or hematopoiesis—is a paradigm for the proliferative and developmental potential of undifferentiated 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, the cloning and molecular characterization of red-cell membrane proteins, studies on the regulation of platelet production, research on the immunologic regulation of hemopoiesis, the use of transgenic mouse systems to study hemoglobin disorders such as sickle cell anemia, and efforts to understand the hematologic and immunologic consequences of malarial parasite infection.

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. Pronuclei are injected with a cloned fragment of human DNA that contains the hemoglobin-S gene. 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 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 proteins, the mutant form of hemoglobin responsible for sickle cell anemia, but there is no sickling of their blood cells. The interaction between a mutated beta and an alpha chain leads to pathological polymerization in humans. In mice, however, human beta-S chains interact with alpha 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.

Another dramatic finding of the past year was the discovery that the severe anemia responsible for death from acute malaria is caused in large part by a protein, tumor necrosis factor (TNF), produced by the host's own immune system.

Malarial anemia can thus be reversed in mice by giving infected individuals an antibody to TNF, a protein synthesized by macrophages. Also, we showed that administering TNF to healthy mice leads to a reduction in erythrocyte precursors. Extension of this work to humans would have dramatic health implications, since malaria strikes between 200 and 300 million people each year, killing about 1.5 million.

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 diffraction data. Because of the high scattering cross section for electrons, this method also offers a unique opportunity to study membrane proteins in a native lipid bilayer environment. Also part of our efforts are studies using video-enhanced light microscopy and (with members of the Chemical Biodynamics Division) light microscopy using linearly or circularly polarized light. Among applications of the former technique are studies of intracellular organelle transport, where organelles can be seen as they move along individual microtubules.

The outer membranes of Gram-negative bacteria, such as *E. coli*, contain a high density of pore-forming proteins, which are often called porins. One such protein, *PhoE* porin, is expressed under conditions of phosphate starvation and has been shown to favor the transport of phosphate-containing compounds and negatively charged molecules. We previously reported the three-dimensional structure of this protein, at a resolution of about 18 Å, and we proposed a 3-D model of *PhoE* porin (see Figure 15). Each funnellike structure in our proposed model is thought to comprise a large water-filled vestibule, leading to a narrower extension. The vestibule wall is formed by ß-sheet, with a large fraction of the ß-strands perpendicular to the membrane plane.

We have now recorded high-resolution images of trehalose-embedded *PhoE* porin that dramatically support our proposed model. The predominant feature of the reconstructed images (also shown in Figure 15) is a trimer of ringlike structures. Each "ring" is the end-on projection of the ß-sheet that

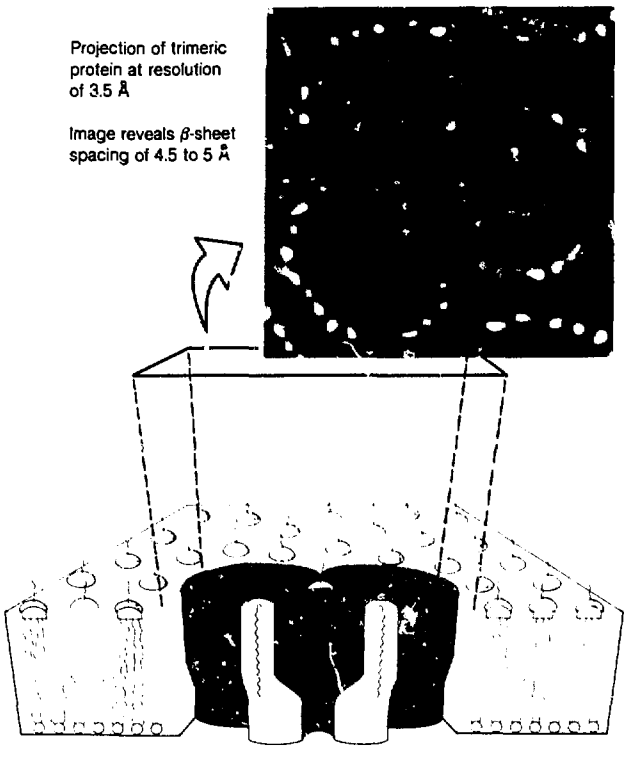


Figure 15. A proposed three-dimensional model for the bacterial pore-forming protein PhoE *porin, together with a reconstructed high-resolution image showing the protein's trimeric structure. The model was proposed on the basis of lower-resolution data; the latest data, which provided the projected image at a resolution of 3.5 Å, supports the picture of pore walls constructed from parallel strands of ß-sheet. [XBB 893-1467 and XBL 886-2204A]*

forms the vestibule wall. The rings consist of "beads" with an interbead spacing of about 4.5–5 Å, which is consistent with the 4.8-Å spacing between strands of a ß-sheet. These beads can therefore be interpreted as projections of the ß-sheet strands along the strand axis. At the center of the trimer, there is a low-density region that has been proposed to be the site of lipopolysaccharides.

We have also made progress toward similar results with other membrane proteins. We recently solved the specimen flatness problem that has hindered work with bacteriorhodopsin, and we have begun collecting images from highly tilted specimens. Using this data, we will soon be able to reconstruct a high-resolution three-dimensional map, in which, for the first time with electron microscopy, the path of the polypeptide chain will be visible. Crystallization efforts have also begun on the adenyl nucleotide transporter from beef heart, H^+-ATPase from plant cells, and halorhodopsin, a light-driven chloride pump.

LIPOPROTEINS AND ATHEROSCLEROSIS

A growing program at LBL focuses on the structure and metabolism of plasma lipoproteins and their relationship to the development of coronary artery disease. Because of their reliance on the techniques of molecular genetics, these studies were carried out in the Cell and Molecular Biology Division through 1988. They now continue in the Molecular Medicine Group of the Research Medicine and Radiation Biophysics Division. The studies focus mainly on the physical and chemical characteristics of the several major classes of lipoproteins, together with their genetic determinants, cellular origins, and metabolic transformations.

The complex relationships among the major lipoprotein subclasses is well-illustrated in Figure 16. Furthermore, many features of this scheme remain incompletely understood, and it does not yet incorporate all the factors that have been implicated

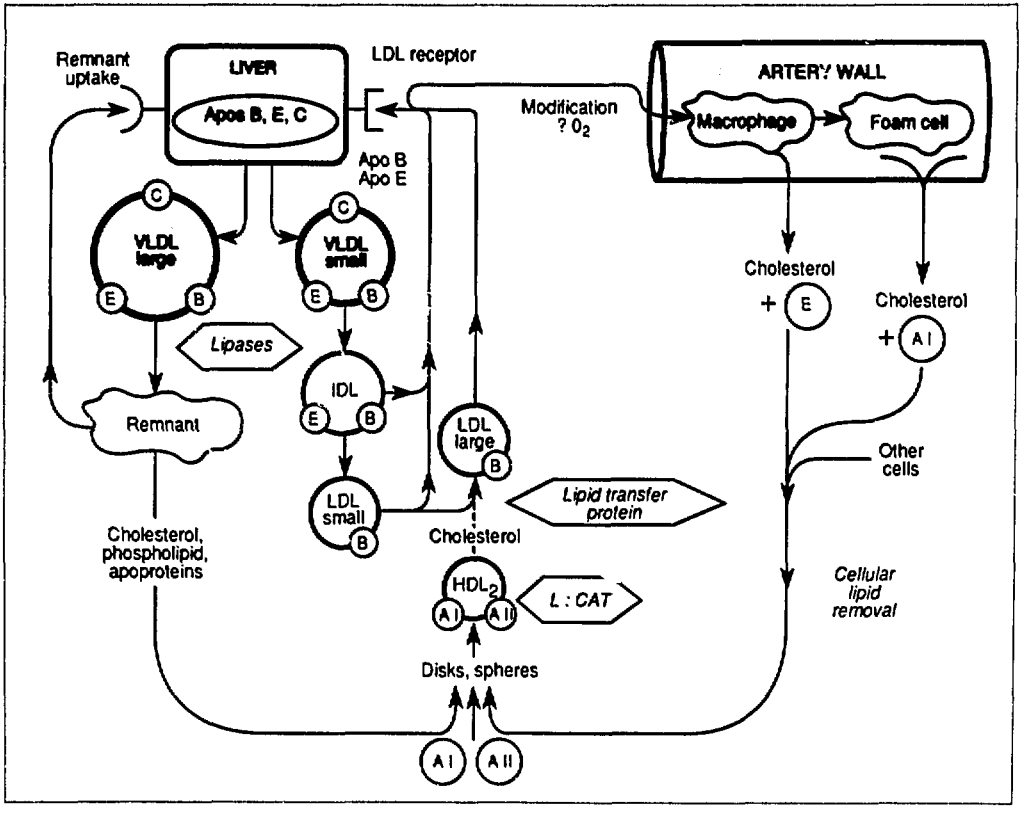


Figure 16. A schematic drawing of the major lipoprotein pathways involved in atherosclerosis. Hydrolysis of triglyceride-rich VLDLs produces smaller particles, including intermediate-density lipoproteins (IDLs) and low-density lipoproteins (LDLs), that transport most of the blood's cholesterol. LDLs that are abnormally modified in the blood are thought to be taken up by arterial macrophages, which then give rise to the foam cells that are the beginning of the atherosclerotic lesion. The HDLs, the so-called good lipoproteins, associated with apoAI and apoAII, are believed to remove excess cholesterol from cells with the help of apoE. [XBL 897-7665]

Abstract

A solution of the well response to a prematurely terminated slug test (PTST) is presented. The advantages of a PTST over conventional slug tests are discussed. A systematized procedure of a PTST is proposed, where a slug test is terminated in the midpoint of the flow period, and the subsequent shut-in data is recorded and analyzed. This method requires a downhole shut-in device and a pressure transducer, which is no more than the conventional deep-well slug testing. As opposed to slug tests, which are ineffective when a skin is present, more accurate estimate of formation permeability can be made using a PTST. Premature termination also shortens the test duration considerably. Because in most cases no more information is gained by completing a slug test to the end, the author recommends that conventional slug tests be replaced by the premature termination technique.

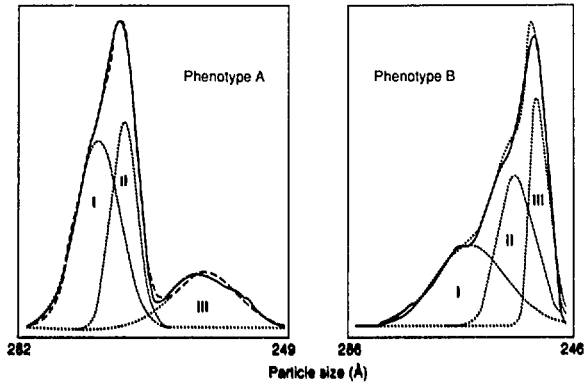


Figure 17. *Gradient gel electrophoresis patterns for two distinct patterns of LDL subclasses. These subclasses, designated I, II, and III, differ in size, density, and lipid and protein composition. Subclass pattern B, which is produced by a dominant allele at a single genetic locus, is linked to a significantly increased risk of heart attack due to atherosclerosis. [XBL 897-7666]*

nated *ALP* (Atherogenic Lipoprotein Profile). The increased coronary disease risk predicted for individuals carrying this gene has already been confirmed by a case-control study showing a two- to threefold increased risk for acute myocardial infarction in individuals with LDL subclass pattern B.

The biochemical basis for the differing LDL subclass patterns is, as yet, not understood. One hypothesis suggests that phenotype B is associated with an altered carbohydrate synthetic pathway in the liver, which may in turn be linked to variations in hepatic LDL metabolism. A second possibility is that "quantized" LDL size transformations are the result of the dissociation of apoproteins and lipids from LDL particles. Both of these models are currently under investigation.

Other studies are likewise aimed at ultimately understanding the relationships of fatal coronary heart disease to lipoprotein subfractions and other established risk factors. One such is a prospective epidemiologic study of 1961 men and 423 women who were employed at the Lawrence Livermore National Laboratory between 1954 and 1957. Our study will extend earlier investigations to 30 years of follow-up. Preliminary analyses suggest that strong relationships exist between lipoprotein subfraction measurements and the subsequent development of heart disease and stroke. Another longitudinal study involves a distinct lipoprotein species, designated Lp(a), whose relationship to coronary heart disease has been studied for over 25 years. The relationship of Lp(a) and other lipoprotein subfractions to the progression of coronary artery disease was examined in collaboration with David Blankenhorn and colleagues at the University of Southern California School of Medicine. Analyses were carried out on 39 subjects, who participated in a two-year trial of drug vs placebo treatment for hypercholesterolemia. In the 19 patients who received drug therapy, Lp(a) concentrations were highly correlated with coronary artery disease progression. A high-molecular-weight

and high-density subfraction of Lp(a), for which the correlation was strongest, thus appears to be a good predictor of atherosclerosis progression in individuals undergoing vigorous drug therapy for hypercholesterolemia.

HUMAN GENOME CENTER

In the three years since early 1986, the "human genome project" has emerged from relative obscurity to a place of unique prominence in the minds of both biologists and laymen. It is a project that has gained a widespread scientific commitment unprecedented in the life sciences community, but 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 only with the beginning of fiscal 1988, when the DOE designated LBL and the Los Alamos National Laboratory as Human Genome Initiative research centers and provided funding for multidisciplinary research. LBL's Human Genome Center was our response to this mandate. Its first year was marked by the appointment of Charles R. Cantor as director, by a proposal for a critically needed new laboratory building, and by dramatic evidence of the importance of coordinated efforts by engineers and computer scientists, working together with biologists.

The Human Genome Project: A Short History

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 base pairs. 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 base pairs 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.

The birth of the "human genome project" can be traced to a 1986 gathering in Santa Fe, New Mexico, where scientists met to ponder the feasibility of sequencing the human genome by the year 2000. The overwhelming, and somewhat surprising, consensus was that such a goal was not only meritorious, but also within practical reach. The U.S. Department of Energy, which had sponsored the Santa Fe meeting, responded to its outcome by announcing in April 1986 preliminary plans for a Human Genome Initiative. In the fall of 1987, the availability of funds for this Initiative signaled a national commitment to the project, and the Department designated the Lawrence Berkeley Laboratory and the Los Alamos National Laboratory as Human Genome Initiative research centers. By September 1988, the National Research Council and the Congressional Office of Technology Assessment had released reports endorsing the effort to map and sequence the human genome, and the international Human Genome Organization had been established to coordinate worldwide efforts. Within three years, a vision had become an acknowledged national, even international, priority.

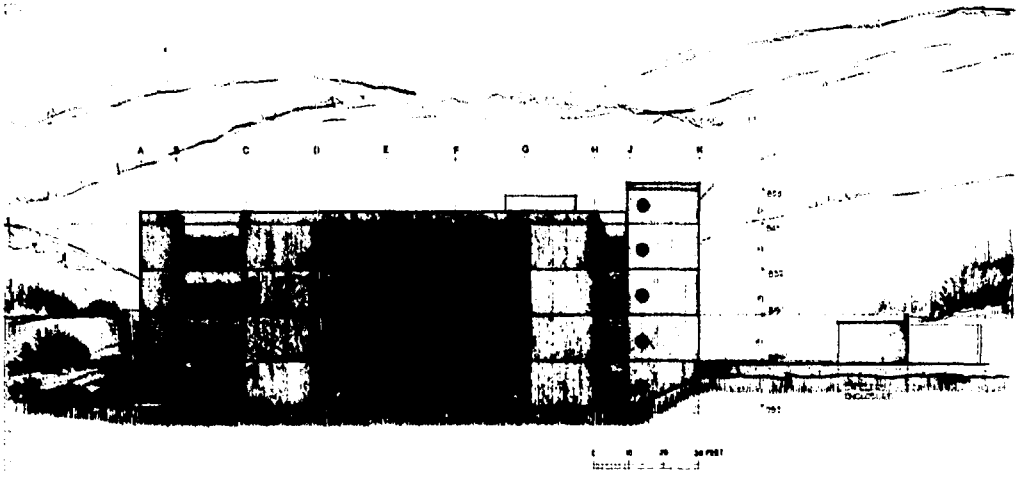
Work at LBL is thus only part of a broad international effort that will dramatically enhance our understanding of human molecular genetics before the end of this century. In this country, the bulk of the effort will be be carried out under the auspices of the Department of Energy and the National Institutes 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 project will ultimately rely on are thus likely to be developed at laboratories like LBL.

Accordingly, current DOE efforts aim primarily at "resource and technology development." This recognizes the current state of technology and the realistic needs of a full-scale mapping effort. With the tools now available, mapping the entire human genome at high resolution would be a painfully slow, labor-intensive, and inordinately expensive undertaking. As a consequence, the emphasis today is twofold. First is the development of analytical and computational resources: better ways to physically manipulate large fragments of DNA, fast and economical (read "automated") methods for ordering these fragments, and a greater facility for storing and handling the resulting huge quantities of data. Second is the actual construc-

tion of an ordered library of cloned DNA fragments. The sum of these fragments, each originating in a human cell but propagated as a clone by genetic engineering techniques, would constitute a reference human genome. With the technological tools in hand and with the subject of study readily available, a full-scale mapping effort could then begin—even an effort to read off the sequence of the three billion base pairs that constitute "the reality of our species."

Much of the necessary interdisciplinary team was in place at LBL by year's end. Contributors to the ongoing effort included members of five LBL research divisions—Cell and Molecular Biology, Chemical Biodynamics, Engineering, Information and Computing Sciences, and Materials and Chemical Sciences—as well as several University of California campuses. Of paramount importance, Charles R. Cantor, a molecular geneticist of international stature, was named to direct the effort. Members of the Center, however, remained dispersed, pending construction of a home for their efforts. A Human Genome Laboratory, sketched in Figure 18, has thus been proposed to provide not only the physical space for this growing 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, 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.

Figure 18. 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 and would ensure a critical interactive mass of researchers in engineering and the physical and biological sciences. [CBB 886-6491]



Early Achievements

In broad terms, the Center's activities are concentrated in three areas: automation of existing physical mapping methods and development of new ones, evaluation and enhancement of existing sequencing technologies, and improvement of methods for interpreting and analyzing maps and sequence data. Current efforts focus on two of the human chromosomes, numbers 21 and 22. Two principles guided the development of this research agenda. First was the strong conviction that methodological development must be accompanied by actual mapping and sequencing efforts. Hence, our program will continue to be characterized by "data-producing" projects, tightly coupled with efforts to refine laboratory methods and to develop new techniques for data handling and data analysis. The second guiding principle was the expectation that powerful automated methods will be absolutely necessary if we are to merge and reconcile research results from the many laboratories that will ultimately be involved in genome mapping and sequencing.

A major goal at the Center is to construct an ordered library of DNA fragments from human chromosomes 21 and 22. In a sense, this effort is the central project at the Center, serving as a focus for efforts to improve the tools of the trade and to develop new ones. A map of restriction enzyme–cutting sites on chromosome 21 is already well-advanced, and our future efforts will complement and build on the research already complete. Previous research has also established the approximate loci for a number of genes on chromosomes 21 and 22, so ordered libraries will point the way naturally to mapping efforts of even higher resolution that focus on chromosomal regions of special interest.

Producing an ordered library of clones requires "linking" the many cloned fragments—that is, establishing which fragments are connected to which in the intact human chromosome. One way to establish linkage is to sequence several hundred base pairs at the ends of cloned restriction fragments, as well as the entire lengths of overlapping linking clones, then to look for matches. We plan to carry out this kind of DNA linking on chromosome 21 by means of automated sequencing procedures and gel readers, thus testing some of the tools that need the most improvement if a full-scale sequencing effort is ever to be undertaken.

Pulsed-field gel electrophoresis (PFG) is one of the central laboratory tools of the human genome project. It recent years, it has become the universal means for separating DNA fragments of up to 10 million base pairs. To reduce separation times and enhance resolution, we have constructed a PFG test bed (Figure 19) that allows conditions such as electric field strength and direction, acidity, and temperature to be monitored and recorded at different places in the gel. Analysis of these quantitative data, never before available, will provide an understanding of the physical basis of the separation and offer a route to optimizing the technique. In addition, using a computer controller, the PFG

Figure 19. A pulsed-field gel electrophoresis test bed. In this PFG test bed, conditions at any point in the gel can be monitored by a probe mounted on a computer-controlled platform (see inset). The result has been data never before available on variations in the electric field, gel temperature, and acidity, among other parameters. The test bed also allows active computer control of electrode potentials, as well as the capability for programming complex pulse cycles. The results of continuing studies with this and other test beds will be increased resolution and shorter separation times, especially for DNA fragments of more than five million base pairs. [CBB 8810-10049 and CBB 893-1881]

test bed allows active control of numerous electrode potentials, together with the capability to program complex pulse cycles.

During the past year, we also made significant strides in reducing the human effort that will be required in some of the historically labor-intensive elements of genetic mapping, namely, the analysis of electrophoresis images, the cross-correlation of results from different experiments, and map construction from electrophoresis data. As shown in Figure 20, for example, we began to apply advanced image-enhancement and image-analysis techniques to the problem of reading electrophoresis gels. In addition, we began work on both a "laboratory notebook" and an "image data base" to serve as resources for eventual automated map construction. And finally, we started work on the software for this final step of automation.

Automated methods will also be needed to ensure a coordinated means for handling and sharing the enormous quantity of data that the genome project will produce, and to evaluate, maintain, and distribute the archives of clones that will be the basic experimental resource for genomic research. As a

Figure 20. Autoradiograms from large DNA fragments separated by gel electrophoresis, before (a) and after (b) digital image enhancement. In these images, the three dark lanes contain standards, which overwhelmed the response of the recording film, whereas the dilute samples produced faint or undetectable bands in the other lanes. The enhanced image shows resolved bands in the standards lanes, as well as quantifiable bands in the sample lanes. [XBB 893-1966 and XBB 893-1967]

start toward answering the first of these needs, we began development of a workstation that we hope will provide a uniform means of data access for all involved in the human genome project. As a logical extension of our electronic laboratory notebook, we designed the workstation as a means for integrating all existing data bases, the result of which could then be displayed hierarchically at different levels of resolution.

During the past year, we also recorded notable achievements along more exploratory lines. Two of the most dramatic suggested that someday we may be able to work with, perhaps even sequence, individual DNA molecules. A cloning technique recently developed by Cetus Corporation, the polymerase chain reaction, is most easily carried out if the desired fragment can be isolated and purified. Thus, the challenge is to obtain the pure fragment one wishes to replicate. One approach is to develop tools for orienting and slicing isolated DNA molecules. To this end, we succeeded in imaging single fluorescently labeled DNA molecules in the light microscope and in manipulating them in soft gels by means of electric fields. Further work will aim at cutting molecules at prescribed sites by methods that include locally delivered restriction enzymes, focused x-rays, and mechanical cleavage.

The second dramatic development revolved around the scanning tunneling microscope, or STM. By passing a fine stylus over a sample on a conductive substrate and monitoring the amount by which the stylus tip must be retracted to maintain a constant current through the sample, the STM has provided topographic images of material surfaces at unprecedented resolutions. In 1988, in a collaboration between scientists at LBL and the Lawrence Livermore National Laboratory, this technique was used to image a single, unstained DNA molecule (Figure 21). Is it possible that with the STM we will someday be able to "visually" read DNA sequences? Nobody knows, but the pursuit of such promising technologies is an essential element in the quest to unlock the secrets of the human genome.

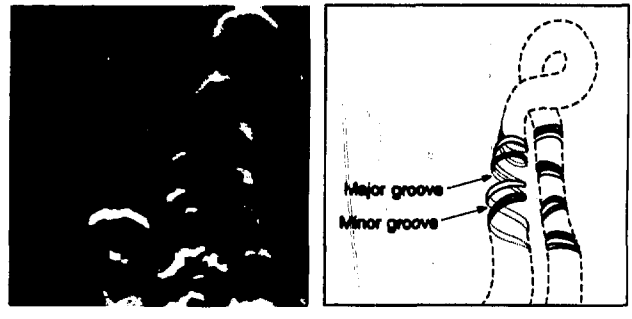


Figure 21. *An image of native, unstained DNA obtained by scanning tunneling microscopy, together with a schematic depiction of the molecule. This image, obtained under conditions of normal atmospheric pressure, was the result of a collaborative effort with the Lawrence Livermore National Laboratory. The image is sufficiently resolved to show the major and minor grooves of the DNA double helix, and some researchers have even suggested that with further development STM imaging may someday be used to sequence DNA. [XBB 880-9840 and XBL 8812-4094]*

Cell and Molecular Biology Division Staff

Mina J. Bissell, Division Director
Michael S. Esposito, Deputy Division Director

Faculty Associates

Bruce Ames
George Brecher
Heinz Fraenkel-Conrat
Michael Freeling
Howard Lecar
Robert Mortimer
William Owen
John Owicki
Roger Tsien

Faculty Senior Scientists

Edward Alpen
Alan Bearden
Robert Glaeser
Alex Nichols
Cornelius Tobias

Senior Scientists

Mina Bissell
Aloke Chatterjee
Gisela Clemons
Michael Esposito
Trudy Forte
Ronald Krauss
Frank Lindgren
Macros Maestre

Staff Scientists

Melissa Austin
John Bartley
Edward Berry
Guy Besson
Eleanor Blakely
Frederick Burkard
Priscilla Cooper
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John Game
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Elaine Gong
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Bea Singer
Thomas Slone
Sylvia Spengler
Martha Stampfer
Thomas Swain
Allan Tischler
Karen Vranizan
Paul Williams
Tracy Yang

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David Chappell
C. Rebecca Contopoulou
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Sarah Hake
Yoshifumi Higashi
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Warren Murray
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Ralph Nossal
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Jaswinder Singh
Jon Stella
John Stubbs
Lynn Verhey
Esther Witte

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Elizabeth Spangler
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Research Associates

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Annie Pang
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Patricia Powers-Risius
Tracy Ram
Brenda Ringel
Janét Selmek-Halsey
Jonathan Slack
Amy Smoll
Michael Wong

Research Associates (Guests)

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Deverie Bongard
Betsey Cullen
Deborah Farson
Anders Greenwood
Mark Hertle
Barbara Lane
Christine Lekutis
Eva Penzes
Neelima Sinha
Sherrie Stevens
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Research Clinical Lab Technologists

Mary Cabbage
Adelle Cavanaugh
Marcia Somers

Technical Assistants

Queen Gipson
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Janis Scherer
David West

Graduate Students

Li-How Chen
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Kyung-Dall Lee
Steven Neben
Michael Sieweke
Peter Walian
Yue Wu
Karen Zetti

Graduate Students (Guests)

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George Basile
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Minh Dang
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Ross Ramos
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Shirley Sutton
Vladimir Vacata
David Warland
Monroe Whitney
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Student Assistants

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Saman Arbabi
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Srisanai Pattamakom
Denise Peterson
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George Serghiou
Usha Srinivasan
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Student Assistants (Guests)

Paul Abdul-Malak
John Baker
Sindy Berger
Paul Brakeman
Peter Breder
Steve Chi-Long Chen
Chao-Hstung Cheng
Victoria Chien
Dongsuk Choi
Andrew Cohen
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Sally Tubach
Francis Wilcox
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Consultants

Leslie Bernstein
Charles Cantor
Howard Ochman

Cell and Molecular Biology Division Publications

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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 has made rich contributions to the understanding of the molecular mechanisms of photosynthesis and the effects of environmental pollutants on plant and animal cells. The study of photosynthesis continues to be a central theme of the Division, as it has been throughout its history, but increasing effort in this area is turning to the use of molecular genetic methods to probe questions of structure and function.

In a second area, both NMR and x-ray crystallographic techniques are being used to probe the molecular 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 not only the primary structure, but also higher-order structure of normal and damaged nucleic acids, including interactions with proteins and with other nucleic acids, regulate the functional activity of genes. In our work on catalytic enzymes, we have even been able to tailor the structure of proteins so that the molecules perform predetermined functions.

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 photon energy, 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

". . . both NMR and x ray crystallography are being used to probe the structure of biomolecules, and the tools of modern molecular biology are being used to explore the broad questions implied by the term 'structural biology'. . . ."

one of the few facilities in the nation equipped to label compounds to very high specific activities of ^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. Among the NTLF's most important activities are to supply labeled biomolecules for tritium-NMR spectroscopy, a key technique in our structural biology research.

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 (see Figure 22). Ongoing studies focus on the kinetics of 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, by seeking to sequence the genes responsible for synthesis of the photosynthetic apparatus, to elucidate the means by which they are regulated, and to explore the activities of the enzymes responsible for pigment biosynthesis. Still other studies aim at a broader understanding of plant biochemistry. In one study, our attention focuses on the biological mechanisms that control hydrocarbon production in plants—plants that may offer an alternative to fossil fuels.

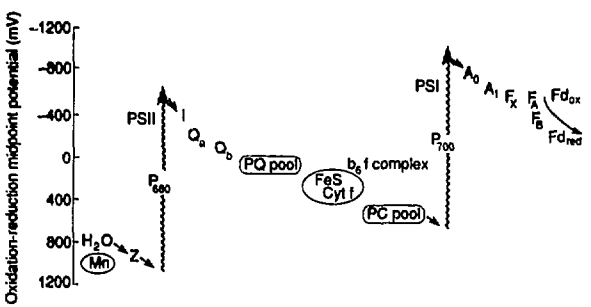

Figure 22. *A diagram of the photosynthetic "Z-scheme," representing noncyclic electron flow in higher plants. Electrons flow from the water oxidation side of photosystem II to the ferredoxin reduction side of photosystem I. The vertical position of each redox-active membrane component represents its approximate midpoint potential at neutral pH. The two vertical lines represent the two light reactions of photosynthesis. [XBL 897-7667]*

The Reactions of Photosynthesis

The conversion of sunlight to chemical energy in green plants is our principal source of biological energy. 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 the photosynthetic pigments, including the earliest steps of electron transfer in the reaction centers and the mechanism by which water is oxidized to molecular oxygen. During the past year, we continued to study fast kinetics by means of optical and electron paramagnetic resonance spectroscopy, as well as using EPR and x-ray absorption spectroscopy to study structural features. We also looked at the higher-order structure and composition of photosynthetic complexes and thylakoid membranes, using biochemical and molecular genetic techniques. And finally, we investigated several model compounds capable of the light-induced electron transfer that lies at the heart of photosynthetic energy conversion, and at compounds that are candidates for storing oxidizing equivalents in the water-oxidation reaction.

As an example of this interrelated program of research, several significant findings emerged from our studies of manganese and water oxidation. It is known that a complex containing four manganese atoms is involved in the storage of the four oxidizing equivalents needed in photosynthesis to produce O_2 from water. To study this complex and the process of oxygen evolution, we have pioneered the use of x-ray edge spectroscopy and EXAFS in studies of manganese, in conjunction with low-temperature EPR measurements. EXAFS studies, in fact, were the first to indicate the presence of a bridged binuclear manganese structure in the oxygen-evolving complex. We have now obtained EXAFS spectra for photosystem II particles from spinach, stabilized in the S_0, S_1, S_2, and S_3 states (see Figure 23), thus considerably illuminating the structure of the manganese complex and the changes that occur during the enzymatic cycle. Further, comparison of photosystem II complexes from spinach and the thermophilic cyanobacterium *Synechococcus* sp, 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

The photosynthetic reaction centers perform the primary photochemistry of photosynthesis. In plants, eucaryotic algae, and cyanobacteria, one of the two reaction centers, photosystem II, utilizes light energy to drive the oxidation of water. The "core" of photosystem II comprises a small number of polypeptides, including polypeptides D_1 and D_2 and cytochrome *b*559, which make up the smallest complex capable of primary

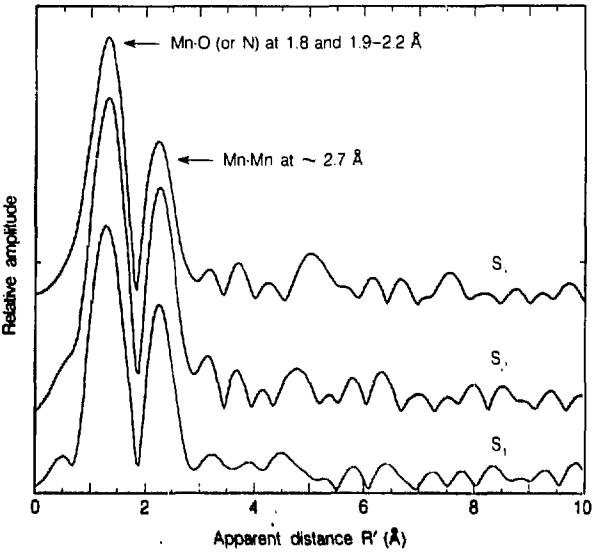

Figure 23. Fourier transforms of the k^1*-weighted manganese EXAFS data from spinach photosystem II samples in the* S_1*,* S_2*, and* S_3 *states. The first peak is due primarily to bridging O (or N) ligands at about 1.8 Å and terminal O (or N) ligands at 1.9–2.2 Å. The second peak arises from manganese at about 2.7 Å. The peaks are characteristic of a di-μ-oxo-bridged manganese cluster. The peaks appear at an apparent distance R′, which is shorter than the true internuclear distance. [XBL 897-6561]*

charge separation. Associated with the photosystem core are chlorophyll-binding proteins, designated CP43 and CP47, which likely act as the core light-harvesting antennae. Additional proteins, bound on the oxidizing side of the membrane, help stabilize the manganese cluster discussed in the preceding section. Much of our ongoing work is aimed at characterizing the structure and function of these several polypeptides.

A transformable strain of cyanobacteria, *Synechococcus* sp PCC 7002, in which one can modify or delete photosystem II genes, has served as the basis for many of our molecular genetic studies. By the end of 1988, we had cloned two of the three genes that code for the D_1 polypeptide, both genes that code for D_2, and single genes that code for *b*559, CP43, and CP47. Our gene-mapping work during the past year has demonstrated that these genes are not clustered on the cyanobacterial genome. We also sequenced the two D_2 genes and showed that they encode identical polypeptides, though the proteins may be expressed or modified differently under different environmental conditions.

Other molecular genetic studies focus on a second bacterium, the photosynthetic species *Rhodobacter capsulatus*. The genome of *R. capsulatus* contains a single region, 46 kilobases long, 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 the biosynthesis of bacteriochlorophyll and carotenoid pigments. Among our aims in studying this genome cluster are to complete the nucleotide sequence, to characterize the activities of the pigment-synthesis enzymes, and to understand how the

genes are regulated. *Rhodobacter capsulatus* is an ideal organism for such studies: In the absence of oxygen, it develops an extensive cytoplasmic membrane system that contains the photosynthetic apparatus, and it is capable of respiratory growth, thus allowing us to maintain and propagate photosynthetic mutants.

One highlight of these studies during 1988 was the sequencing of 11,039 base pairs of the *R. capsulatus* genome, containing seven of the eight previously identified carotenoid genes (Figure 24). Despite the crucial role of these pigments in photooxidative protection, no carotenoid gene from any organism had previously been sequenced, nor had the molecular mechanisms regulating carotenogenesis been elucidated. After obtaining the sequence, we were able to identify a previously unknown gene, now designated *crtK*, and we have identified possible promoters (similar to those found in the bacterium *Escherichia coli*) and a possible binding site for regulatory proteins.

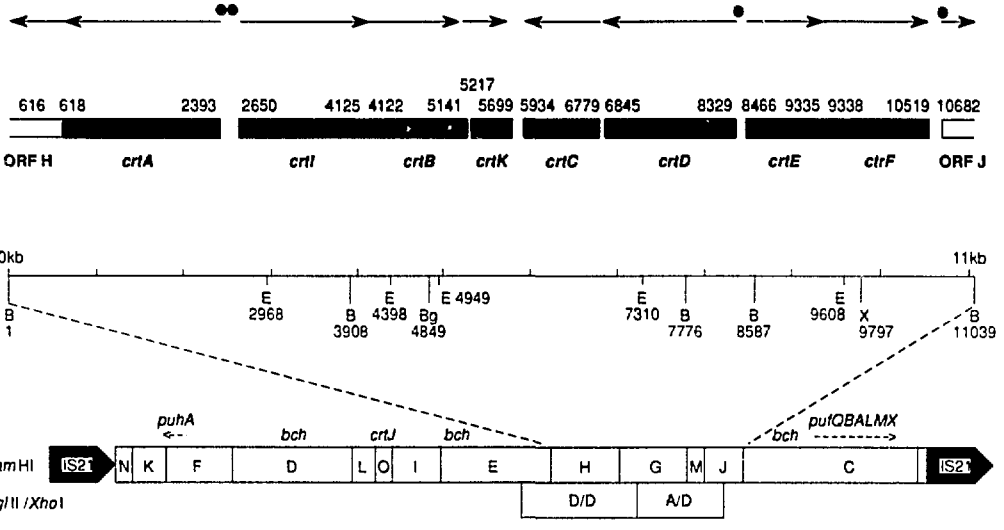

Figure 24. *A schematic diagram showing the organization of the carotenoid* (crt) *biosynthetic gene cluster in* Rhodobacter capsulatus. *Directions of transcription are indicated with arrows, and putative regulatory sites are indicated by dots. Numbers above the genes show the putative nucleotide positions of translational starts and stops. Restriction sites are indicated below the genes:* B, BamHI; E, EcoRI; Bg, BglII; X, XhoI. *Boxes containing letters indicate specific restriction fragments from the photosynthetic gene cluster, as determined in earlier work; the IS21 elements derived from the vector are also shown. The locations of photosynthetic genes outside the* crt *cluster are shown above the boxes; these genes code for various light-harvesting and reaction-center polypeptides, as well as for enzymes that catalyze synthesis of bacteriochlorophyll. [XBL 897-6562]*

Regulation of Gene Expression

Whereas photosynthetic processes are carried out primarily in the chloroplasts of eucaryotes, some of the genes encoding the necessary proteins are found only in the nuclear genome. Little is known about the mechanisms that coordinate the expression of these genes with the expression of genes in the chloroplast genome. In most eucaryotic cells, the nuclear pattern of gene expression is at least partly linked to the position of the cells in the cell cycle. In the eucaryotic alga *Euglena gracilis*, light has a dramatic effect on cell cycle traverse, and it may therefore affect

gene expression simply by affecting the cell cycle. In one of our studies of gene expression, our aim is to explore the regulatory effect of light in *Euglena*.

Using probes for several genes associated with the photosynthetic apparatus, we have now found a light-dependent accumulation of mRNA in *Euglena*, even when the organism is grown to saturation density, which inhibits cell cycle traverse. On the other hand, gene expression is more pronounced in cells that are not thus inhibited. These results suggest that the genes for components of the photosynthetic apparatus are regulated by light, independent of the cell cycle, but that the progress of cells around the cycle can affect the message level quantitatively. We further showed in mutant *Euglena* lacking chloroplasts that the cell cycle is regulated by nutrient levels in the heterotrophic medium, rather than by light.

In a second study on gene expression, we are continuing to look for the cellular factors responsible for tissue-specific expression of *Agrobacterium rhizogenes* Ri T-RNA in potato and tobacco plants.

Plant Biochemistry

Hydrocarbon-producing plants are a potential alternative to fossil fuels as a source of both energy and feedstock for industrial chemical processes. We are therefore interested in the biological mechanisms that control hydrocarbon production in plants, especially the production of the isoprenoids. Identification of these control mechanisms may ultimately permit manipulation of the plant genome to increase hydrocarbon yields to practical levels. For our biochemical studies in this area, we use latex isolated from the laticifer cells of *Euphorbia lathyris*. We have centered our studies on three problems: (i) identification of the rate-limiting steps in triterpenoid biosynthesis, (ii) determination of the mechanism by which squalene, an acyclic triterpene, is cyclized to form the triterpenoids, and (iii) identification of the organelles in the latex and determination of their biosynthetic roles. In the first of these research areas, as an example, we have identified the rate-limiting step (the conversion of ß-hydroxymethyl glutaryl coenzyme A, or HMG-CoA, to mevalonic acid), isolated and identified the responsible enzyme, identified and characterized two isozymes, and identified a "competing" enzyme, which inhibits triterpenoid synthesis by breaking down HMG-CoA.

In a second area of plant biochemistry research, we are exploring both structural and functional aspects of the plant protein-pigment phytochrome, the plant pigment that controls all developmental aspects of plant growth. Phytochrome acts by undergoing a photochemical interconversion to a new, active form; however, the structural features of this isomerization, as well as other aspects of phytochrome structure, remain unknown. Among our aims, then, are to determine the stereo-

chemistry of the pigment-protein linkage in phytochrome and to determine the structural changes in both pigment and protein that accompany the change from inactive (P_R) to active (P_{FR}) 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 with predetermined reaction specificities.

NMR Studies

Our use of NMR in the analysis of biomolecular structure includes studies of DNA, RNA, and proteins. As an example, our efforts to determine protein structure in solution are aimed at understanding the basis for their folding patterns, their activity, and their interactions with other molecules. One particularly interesting application is to determine the residues involved in substrate binding and chemical activity in catalytically active antibodies (see also pages 59–60). We have thus developed methods for obtaining difference spectra for different states of active Fab fragments: free fragments from the catalytically active antibodies, Fabs with bound haptens, and Fabs with spin-labeled haptens bound in both oxidized and reduced states (Figure 25). Ongoing studies with antibodies grown on ^{2}H-labeled amino acids will allow us to unambiguously identify the residues of the active site.

We took a similar approach in analyzing the functional role of His 134 in the catalytic activity of aspartate transcarbamylase. We unambiguously assigned the resonance of His 134, then followed it as a function of *p*H. As a result, we showed that the likely role of the residue is not to behave as an acid or a base (both roles had been suggested by others), but rather to assist in

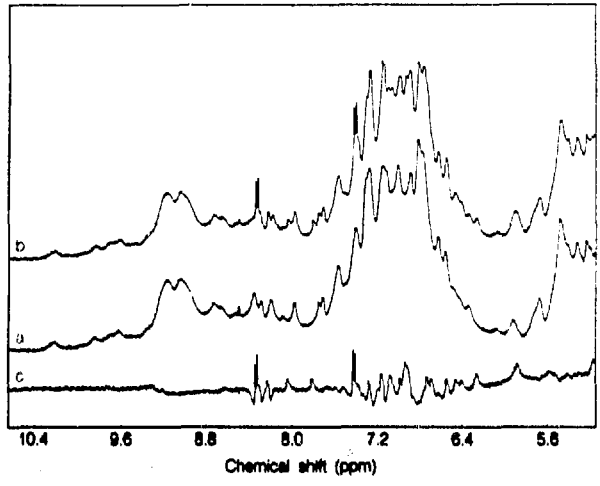

Figure 25. *Proton-NMR spectra showing the amide and aromatic regions of an antibody complex. (a) Spectrum of the antibody with a bound paramagnetic hapten. (b) Spectrum of the same sample after reduction of the hapten nitroxide to a hydroxylamine. (c) Difference spectrum (b – a) showing only peaks near the hapten binding site. [XBL 897-6563]*

the positioning of the substrate.

A second application of NMR spectroscopy is in studies of cellular metabolism in vivo. ^{31}P-NMR, for example, has evolved into an important means for determining in vivo 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 rat hearts and kidneys and demonstrated that there are pools of metabolic intermediates not directly visible in conventional high-resolution NMR spectra.

X-Ray Crystallographic Studies

A key crystallographic result of the past year 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 and normal forms.

The *ras* oncogene is one of the most commonly found oncogenes in human cancer cells. A single point mutation, resulting in production of a protein with a single amino acid substitution, is sufficient to convert the normal *ras* gene product to an oncogenic form. In one such mutant, a valine codon replaces a glycine codon at position 12. The protein produced by the resulting gene, complexed with guanosine triphosphate

(GTP), is thought to function by sustaining 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.

During the last year, we refined the normal *ras* protein structure to a resolution of 2.2 Å. This higher-resolution picture reveals atomic details of the three-dimensional structure (see Figure 26) that will provide the foundation for understanding how the protein functions and for aiding in drug design that may suppress *ras*-mediated human tumors. We are currently refining the structure of the valine-12 mutant at the same high resolution, so as to identify the structural differences between the normal and mutant forms and to understand their functional implications.

Synthesis of Catalytic Antibodies

The ability to "design" and synthesize artificial enzymes with well-characterized chemical properties would be a dramatic advance in the commercialization of many chemical processes, as well as having important biomedical applications. Key to the design of such selective catalysts is the generation of highly selective binding sites. With the advent of monoclonal antibodies, we can now generate such binding sites, with enzymelike affinities and specificities, as parts of antibodies produced by living cells. For example, antibodies have been produced against biopolymers such as nucleic acids, proteins, and polysaccharides; against smaller multifunctional molecules such as steroids and prostaglandins; and against synthetic polymers.

One strategy whereby catalytic antibodies might be generated involves the site-specific introduction of catalytic residues into antibody combining sites. One might accomplish this, for example, by genetic methods, including site-directed mutagenesis and selection, or by selective chemical modification. Using yet a third technique, exploiting a group of antigens known as haptens, we have successfully generated antibodies that catalyze the photocleavage of thymine dimers.

Thymine dimers are the major photolesions that result from irradiation of DNA with UV light. Organisms have

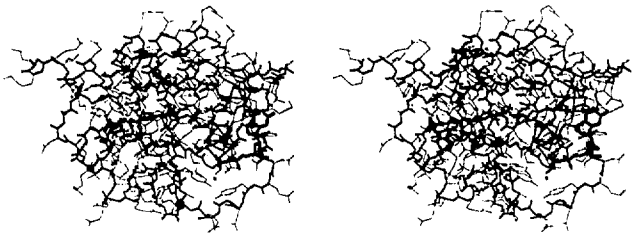

Figure 26. *Stereo drawings of the complete atomic structure of the normal* ras *protein, at a resolution of 2.2 Å. [XBL 897-6564]*

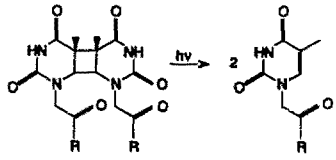

Figure 27. Photochemical cleavage of a thymine dimer. Five thymine dimer–specific monoclonal antibodies have now been isolated that efficiently catalyze this reaction when exposed to ultraviolet light. [XBL 897-5565]

evolved a number of systems to repair thymine dimers, including light-dependent photoreactivating enzymes. The dimer cleavage reaction is shown in Figure 27. Although the mechanism of the enzymatic reaction remains poorly understood, it has been demonstrated in model systems that a number of sensitizers, including indoles, quinones, and deazaflavins, can photosensitize dimer cleavage. One might, therefore, imagine that an antibody combining site, specific for thymine dimers and containing an appropriately positioned sensitizer, might act as a selective photoreactivating enzyme. Indeed, we have isolated and characterized six monoclonal antibodies specific for thymine dimers, five of which efficiently cleave thymine dimers when irradiated with UV light.

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 DNA damage repair, the structure of catalytically active RNA, the mechanisms by which DNA synthesis is controlled in human cells, the structure of left-handed Z-RNA, the structure of telomeric DNA oligonucleotides, and the tertiary structure of RNA "pseudoknots." We have also developed a differential polarization microscope, which reveals ordered linear structures and chiral structures in single live 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, many messenger RNAs contain intervening sequences, introns, that 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.

We are taking several approaches to determining the secondary and tertiary structures of catalytic RNAs. One method is to photochemically crosslink the molecule with psoralen, which stabilizes certain elements of the RNA structure by establishing covalent linkages within duplex regions. 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, 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 empirical energy-modeling computer

program to broadly search plausible regions of conformational space, at the same time minimizing energy to locate the best local conformations. In a preliminary study using this approach, we determined the tertiary structure of the yeast phenylalanine tRNA, based only on knowledge of the primary structure, deduced hydrogen bonding, and available information on five long-range interactions. Subsequent x-ray crystallography results confirmed our conclusion.

Another especially illustrative study focused on the tertiary structure of RNA pseudoknots. The many biological functions of RNA, including its enzymatic activities and its regulatory roles in transcription and translation, are made possible by its tertiary structure. However whereas secondary structure can be predicted by use of free energy parameters, little is known about the tertiary structure of RNA—with the exception of tRNA. We thus undertook a study of pseudoknots, a tertiary structure proposed for the 3′ ends of many plant viral RNAs. This pseudoknotting allows the virus to fold itself so that it looks like tRNA. During the past year, we characterized the pseudoknot conformation, shown in Figure 28, using NMR, absorbance melting curves, single- and double-strand-specific enzymes, and gel electrophoresis. The deduced structure shows that the two stems of the pseudoknot stack atop one another so as to form what appears to be one continuous, longer stem. The stem has the standard RNA double helix structure, explaining why the pseudoknot can fool enzymes that recognize tRNA.

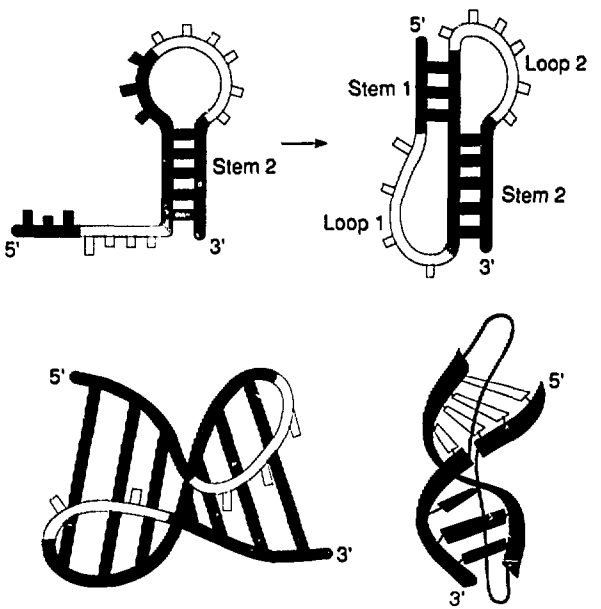

Figure 28. *A schematic illustration of an RNA pseudoknot structure determined by NMR, absorbance melting curves, single- and double-strand-specific enzymes, and gel electrophoresis. The two stems of the pseudoknot stack atop one another to form an apparent single stem; the pseudoknot thus mimics tRNA. [XBL 897-6566]*

$$\bar{I}^2 = 3\Im T + \left[\frac{\Im}{\sum(\Im + mr^2)}\right]^2 I_T^2 . \tag{123}$$

This equation says that the fragment angular momentum arises from two contributions: the first is purely statistical and would exist also for zero angular momentum; the second is the share of the total angular momentum going to the fragment under study, dictated by the rigid-rotation condition. The two contributions are added in quadrature. From the structure of Eq. 123, one would also infer that

$$\sigma_x^2 = \sigma_y^2 = \sigma_z^2 = \Im T , \tag{124}$$

the average for I_x and I_y being zero and for I_z being

$$\bar{I}_z = \frac{\Im}{\sum(\Im + mr^2)} I_T . \tag{125}$$

The latter inference can be verified directly. By isolation of the factor containing I_z in the partition function, one has

$$Z_{I_z} = \prod \exp - \left[\frac{I_z^2}{2\Im T} - \mu I_z\right] . \tag{126}$$

Thus,

$$\bar{I}_z = \frac{\partial \ln Z_{I_z}}{\partial \mu} = \mu \Im T = \frac{\Im}{\sum(\Im + mr^2)} I_T \tag{127}$$

as expected. Consequently,

$$\sigma_x^2 = \sigma_y^2 = \sigma_x^2 = \Im T . \tag{128}$$

The results obtained so far allow us to describe the fragment-spin alignment through the relevant components of the polarization tensor:

$$P_{xy} \propto \sigma_x^2 - \sigma_y^2 = 0 , \tag{129}$$

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

We have directed significant effort toward a fundamental understanding of the special chemistry of electronically excited molecules. We believe that the key to photon energy storage can ultimately be found in the differences between ground-state and excited molecules, in terms of orbital occupancy, charge distribution, molecular structure, and chemical reactivity. A powerful diagnostic tool for investigating these differences is infrared spectroscopy, coupled with matrix isolation. Using this technique, together with a tunable laser photolysis source, we are attempting to map electronic hypersurfaces for both unimolecular and bimolecular reactions.

An example of our progress during 1988 was our work with hydrogen halide–acetylene complexes in cryogenic matrices. The photochemistry is characteristic of an excited "supermolecule," rather than excited individual reactants. Using tuned laser excitation, we saw a strong wavelength dependence of the favored reaction:

$$HI{\cdot}C_2D_2 \xrightarrow[222\ nm]{} DI{\cdot}C_2HD$$

$$HI{\cdot}C_2D_2 \xrightarrow[304\ nm]{} HD{\cdot}C_2DI$$

We are continuing our attempts to understand this dependence in terms of the potential functions of HI and of the supermolecular complex.

A second research effort in basic photochemistry is aimed at identifying chemical reactions that can be initiated with near-infrared photons. Significant solar energy lies in this energy range, thus our studies may establish a basis for practical energy storage, for the conversion of solar to electrical energy, and for the production of useful chemicals. Our tools include time-resolved emission and absorption spectroscopy of redox reactions in aqueous solution, Fourier transform–infrared spectroscopy of reaction intermediates and products in inert matrices, and tuned cw dye lasers as photolysis sources. A key recent result was the observation of unusually high stereo-control in the photooxidation of *cis* and *trans* 2-butene to epoxide when the 2-butene–NO_2 reactant pairs were excited in a matrix with long-wavelength visible light—light of insufficient energy to dissociate the NO_2.

Chemical Biodynamics Division Staff

John E. Hearst, Acting Division Director
George C. Pimentel, Division Director (July 1, 1980 – June 30, 1988)
James C. Bartholomew, Deputy Division Director

Faculty Associates

Ed Bennett
Melvin Calvin
John E. Hearst
Sung-Hou Kim
George C. Pimentel
Henry Rapoport
Kenneth Sauer
Peter Schultz
Ignacio Tinoco, Jr.
David Wemmer

Senior Scientific Staff

James C. Bartholomew
Melvin P. Klein
John W. Otvos

Visiting Faculty

Juana Acrivos
George A. Brooks
Alexander Glazer
Dolon Konwer
Munitaka Nakata
Ning Pon
Rafael Rafaeloff
Michael Saxton
Lawrence Spreer
Stavros Voliotis

Divisional Fellows

Heinz Frei
Francesca Leach

Scientific and Technical Staff

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Duncan Beniston
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Phil Eggers
Stephen Holbrook
Jarmila Jancarik
Rosalind Kim
David Koh
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Gary Smith
Manouchar Saljoughian
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Scott Taylor
Philip Williams
Vittal Yachandra
Hisao Yokota
Yu-Sheng Zhu

Administrator

Lois Soule

Administrative Staff

Mary Bundy
Gloria Goldberg
Beth Klingel
Rosalyn Miles
Evangeline Peterson
Marilyn Taylor

Postdoctoral Fellows

Mohdaslam Ansari
Kazuko Aoyagi
Gier Braathen
Karen Brewer
David Britt
Pi-Tai Chou
Dae Yoon Chi
Carl Craig
Abraham DeVos
Jeffrey Gingrich
Vicki Grassian
Libby Holbrook
Yong-Ki Hong
Deborah Kallick
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Zong-Qi Li
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Pedro Matias
Vasant Nagulappali
Edward Orton
Jay Pandit
Farid Salama
Peter Sandusky
Bethe Scalletar
William Thurmes
Sun Un
Gabrielle Varani
Xinwei Yan
Mark Young
Jean-Luc Zimmerman

Graduate Students

Gregory Armstrong
Enoch Baldwin
Reza Beigi
John Bishop
Donald Burke-Aguerro
Ann Caviani
Michael Chastain
Chae Joon Cheong
Hyunho Chung
Edgar Civitello
Andrea Cochran
James Cole
James Corbett
David Cook
Victoria DeRose
Susan Dexheimer
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Elizabeth Snowden
Peter Spielmann
Richard Storrs
Liang Tong
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Andrew Van Sickle
Niles Warren
Milton Werner
Brent Wurfel
Muh-Ching Yee

Undergraduates

Patty Casey
Johnson Chan
John Kim
Jun Kim
Prudence Lee
Hans Peterson
Ann Williamson

Chemical Biodynamics Division Publications

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5.0. Summary and Conclusions

In the present paper, a solution to a prematurely terminated slug test (PTST) problem was presented. A PTST is, in essence, the same as a drill stem test (DST), which is more widely known in the petroleum terminology. The author wishes to bring attention of field hydrologists to the utility of a DST. A systematized procedure of a DST was proposed, where a slug test is terminated in the midpoint of the flow period, and subsequent shut-in data are recorded and analyzed. This method requires a downhole shut-in device and a pressure transducer, which is no more than the conventional deep-well slug testing. The advantages of a DST over a conventional slug test were discussed. As opposed to a slug test, which are ineffective when a skin is present, more accurate estimate of formation permeability can be made using a DST. Premature termination also shortens the test duration considerably. Because in most cases no more information is gained by completing a slug test to the end, the author recommends that conventional slug tests be replaced by premature termination technique. It is important to note here, however, that for more accurate estimation of aquifer parameters, constant rate tests are much more favorable.

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OCCUPATIONAL HEALTH

LIKE ANY RESPONSIBLE EMPLOYER, THE Lawrence Berkeley Laboratory must be concerned about the health and safety of its work force. This means not only taking account of the usual hazards of the work place, from loose stair treads to major earthquakes, but also ensuring that the tools and materials of modern scientific research—as different and diverse as particle accelerators and oncogenic viruses—are properly handled. Broadly speaking, shouldering this responsibility is the role of the Occupational Health Division.

". . . taking account of the usual hazards of the work place and ensuring that the tools and materials of modern scientific research—as different as particle accelerators and oncogenic viruses—are properly handled. . . ."

The resulting tasks include many "routine" activities, ranging from soil and groundwater sampling to employee safety training. Particularly notable extensions of the latter are periodic earthquake awareness drills, in which all laboratory employees participate, and multicasualty drills designed to exercise the emergency response capabilities of the Laboratory. Also within Occupational Health is the Medical Services Department, whose most visible responsibility is to conduct periodic health examinations of all LBL employees. Another activity closely and obviously related to employee health is the ongoing effort to modernize the personal radiation dosimetry system by replacing all film badges with thermoluminescent dosimeters.

Beyond the routine, however, and setting the Occupational Health Division apart from its industrial counterparts, is the division's work in radiation physics, including applied research conducted on behalf of other DOE accelerator facilities. During the past year, we measured and analyzed neutron spectra at the Bevalac and, on behalf of the SSC, at Fermilab's Tevatron; we designed and evaluated the shielding for the Advanced Light Source, based on radiation transport calculations; and several members of the Division participated in a shielding design and radiation safety review of the Continuous Electron Beam Accelerator Facility under construction at Newport News, Virginia.

ENVIRONMENTAL HEALTH AND SAFETY

The challenge of ensuring employee safety and guaranteeing compliance with community environmental requirements, as well as state and federal statutes, falls largely to the Environmental Health and Safety Department. Broadly, its responsibilities fall into five categories: (i) Industrial hygiene encompasses hazard control programs and a wide range of environmental protection programs. (ii) The operations group is responsible for making monthly surveys of all work with radioisotopes, monitoring radioactive materials and sources, and collecting radioactive and chemical wastes. This group was actively involved in the decommissioning of the 184-Inch Synchrocyclotron and the disposal of 8224 tons of shielding blocks. (iii) Activities of the occupational safety and engineering services group include monitoring construction safety and compiling illness and accident statistics for the Laboratory population. (iv) Training programs in health and safety are provided for all employees. (v) Health physics covers research on radiation physics and accelerator safety, responsibility for radiological environmental monitoring, and coordination of the personal dosimetry program.

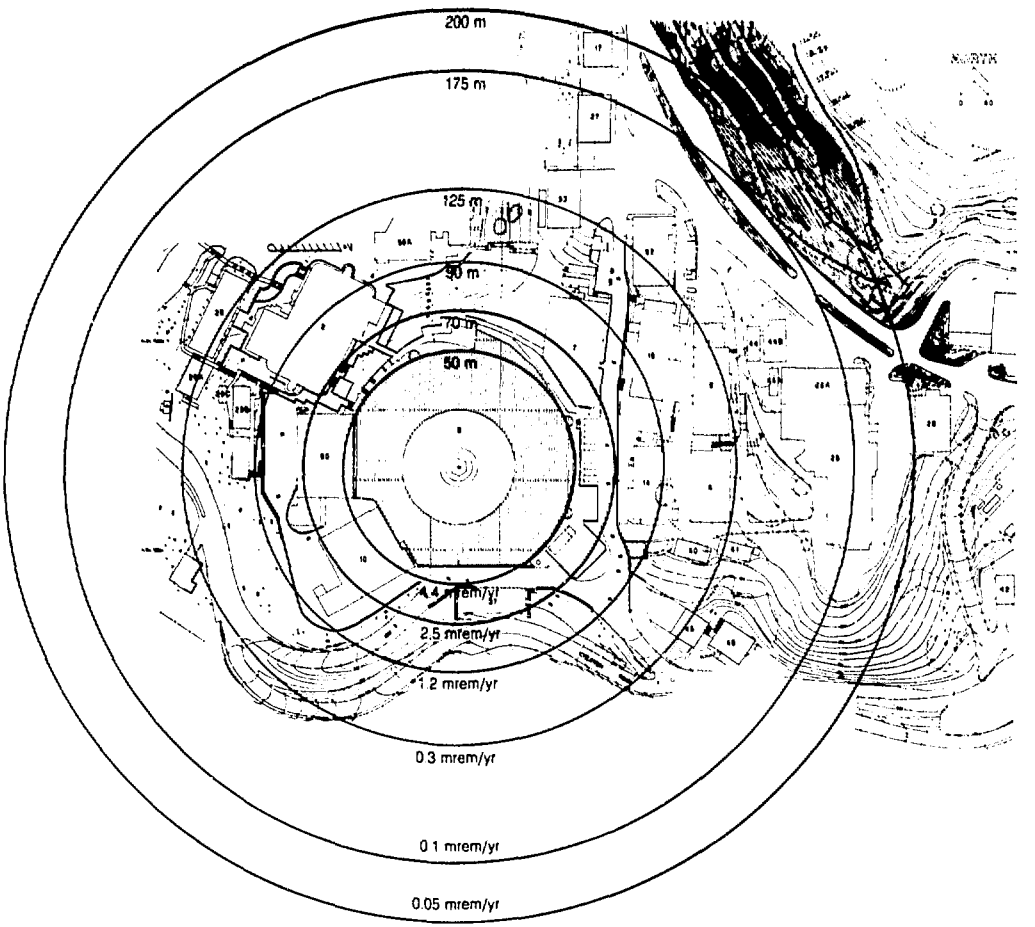
Much that is unique in the realm of occupational health at LBL centers around the presence of its several and varied accelerators, including the Bevalac and the Advanced Light Source (ALS), the latter now under construction. Indeed, the continuous presence of major accelerators at the Laboratory for more than 50 years has led to a considerable local concentration of expertise in radiation physics and accelerator safety. Accordingly, our activities in these fields are not restricted to LBL, but rather involve other national accelerator facilities as well.

Such activities included the continuing analysis of neutron spectra obtained with our multisphere system at Fermilab's Tevatron. Measurements were made in cooperation with Fermilab staff at the request of the SSC Central Design Group, as a way to estimate radiation damage to electronic systems located near high-energy accelerator rings. We also participated in a radiation safety review of the Continuous Electron Beam Accelerator Facility (CEBAF), and carried out a number of calculations pertinent to CEBAF design, including the neutron field attenuation through shielding labyrinths, tritium production in liquid helium, and radioactivation of the machine environment. We also showed that the classic neutron transport code MORSE, developed at the Oak Ridge National Laboratory, predicted air-scattered neutron, or "skyshine," intensities consistent with those predicted by other methods. As a third service to the community, several division members worked with scientists from Brookhaven, Fermilab, and Los Alamos to

produce the *DOE Health Physics Manual of Good Practices for Accelerator Facilities.*

At LBL our radiation physics studies during 1988 focused on the Bevalac and the ALS. The ALS shielding design was previously determined by scaling experimental measurements made at other facilities. One of our efforts, then, was to use the MORSE code to supplement our earlier calculations with calculations for complex geometries. The design goals included minimization of dose-equivalent rates in work areas and in nearby offices, as well as annual dose-equivalents from skyshine neutrons at the LBL site boundary. As shown in Figure 29, our preliminary results confirmed the adequacy of the ALS shielding design. We also reviewed engineering and architectural plans for the ALS, focusing on such issues as radiation transport through labyrinths, beamlines, and beam dumps; doses to electronic components; and the effects of radiation on magnet materials.

Figure 29. *A plan view of the Laboratory site in the vicinity of the Advanced Light Source, showing calculated annual dose equivalents due to air-scattered neutrons, or "skyshine," from the electron storage ring. The calculated dose at the nearest laboratory boundary is a small fraction of the natural background. [XBL 897-6567]*



We measured the neutron energy spectrum at two locations in the Bevalac biomedical control room, using a low-resolution multisphere neutron spectrometer. At the same time, we evaluated the responses of three personal neutron dosimeters: a track-etch plastic (CR-39), a bubble detector, and an NTA film. Problems of sensitivity, accuracy, and precision at low dose levels and in mixed high-energy radiation fields pose particular challenges for such dosimetry systems, and evaluations continue.

MEDICAL SERVICES AND EMERGENCY PREPAREDNESS

Two other concerns of the Occupational Health Division are emergency preparedness, including readiness for always-possible earthquakes, and provision of both routine and emergency medical services. The most visible activity of the Emergency Preparedness Department is supervision of periodic practice drills, whereas medical services are most apparent during routine employee checkups.

Occupational medical services have been available to Laboratory employees for the past 30 years, with special emphasis on the unique working environment of a research facility. As always, our objectives are to ensure physically suitable job placement, to provide care for the occupationally ill or injured, to provide emergency treatment, to encourage employees to maintain their health, and to assist in maintaining a healthful work place. Preplacement physical examinations are required of all employees, and we also offer periodic exams, as well as checkups at the

Figure 30. *A scene during the multi-casualty drill held in the spring of 1988. [XBC 895-3948]*

time of retirement or termination. Of particular importance, special examinations are conducted for laser users, and a bioassay program, coordinated with the personal dosimetry program, is in place for all employees who work with radioisotopes. A safety glasses program is also available. Among less routine activities, the Medical Services Department has also established a mutual aid agreement with Alta Bates Hospital, in the event of a radiation accident at the Laboratory or elsewhere, and during 1988 we instituted a Hepatitis B Vaccination Program to protect employees working with hepatitis-infected blood or blood products.

One important emergency preparedness activity is the training and exercise of emergency response teams. A multicasualty drill was thus conducted during the spring of 1988, not to assess the response of the larger Laboratory population, but rather to evaluate the readiness of building emergency teams, auxiliary ambulance and firefighting teams, medical response teams, public information and amateur radio teams, and all professional emergency response groups. The scenario was an explosion and fire at Building 90, in contrast to a Laboratory-wide emergency such as a major earthquake. As shown in Figure 30, simulated victims added considerable realism to the triage, emergency treatment, and transportation procedures. Impartial umpires, on hand to judge the effectiveness of our response to this simulated event, concluded that the emergency teams were well-prepared, skillful, and competent. At the same time, constructive suggestions underscore the importance of such drills in improving our responsiveness to emergencies.

Occupational Health Division Staff

Ralph H. Thomas, Division Director
Jensen Young, Deputy Division Director

Staff Scientists

Raymond Aune
Peter Bringham
Theodore DeCastro
William Donald
Nathaniel Greenhouse
James Haley
Richard Harvey
Calvin Jackson
Pierre La Plant
Joseph McCaslin
Marshall Ow
Ronald Pauer
Gary Schleimer
Charles Schmidt
Charles Smith
Lisa Snow
Henry Stauffer
Rai-Ko Sun
Ralph Thomas
Jensen Young

Technical Staff

Kenneth Biscay
Nancy Bradfield
Winifred Corniea
Eileen Doyle
Roscoe Drisdale
Pauline Fleischer
Glenn Garabedian
Donald Gregerson
Connie Grondona
Suzanne Hargis
William Hemphill
Jacqueline Ivelich
Harry Jelonek
Judith Kody
Mark Lasartemy
Henrietta Lee
William Letchworth
Yea Liu
Mary Long
Josephine Lundbergh
Victor Montoya
Bette Muhammad
Wayne Pearce
Pope Sealy
David Yeager
Randy Yow

Administrative Staff

Mona Bernstein
Wilma Charles
Joan Creson
Nancy Dietrich
Carol Eaton
Sandi Kronquist
Linda Noel
Roberta Trout
Alice Vdovin
Suzanna Wingerson

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